This Page Is Inserted by IFW Operations and is not a part of the Official Record

BEST AVAILABLE IMAGES

Defective images within this document are accurate representations of the original documents submitted by the applicant.

Defects in the images may include (but are not limited to):

- BLACK BORDERS
- TEXT CUT OFF AT TOP, BOTTOM OR SIDES
- FADED TEXT
- ILLEGIBLE TEXT
- SKEWED/SLANTED IMAGES
- COLORED PHOTOS
- BLACK OR VERY BLACK AND WHITE DARK PHOTOS
- GRAY SCALE DOCUMENTS

IMAGES ARE BEST AVAILABLE COPY.

As rescanning documents will not correct images, please do not report the images to the Image Problem Mailbox.

.... FAUE BLANK (USPTO)



WORLD INTELLECTUAL PROPERTY ORGANIZATION International Bureau

PCT

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 6: C12N 15/62, A61K 39/395, 38/17, 47/48, 51/10, C07K 16/30, 16/46, 16/00, C12N 15/13, 1/21, 5/10 // C07K 19/00

(11) International Publication Number:

WO 98/05787

(43) International Publication Date:

12 February 1998 (12.02.98)

(21) International Application Number:

PCT/US97/13562

A1

(22) International Filing Date:

1 August 1997 (01.08.97)

(81) Designated States: AU, CA, JP, European patent (AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE).

(30) Priority Data:

60/023,033

2 August 1996 (02.08.96)

Published

US

With international search report.

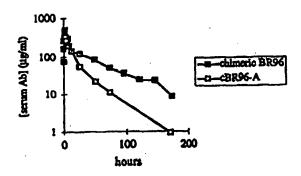
Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.

BRISTOL-MYERS SQUIBB COMPANY (71) Applicant: [US/US]; 345 Park Avenue, New York, NY 10154 (US).

(72) Inventors: ROSOK, Mae, Joanne; 6340 N.E. 194th Street, Seattle, WA 98155 (US). YELTON, Dale, E.; 2307 19th Avenue East, Seattle, WA 98112 (US).

(74) Agent: ADRIANO, Sarah, B.; Merchant, Gould, Smith, Edell, Welter & Schmidt, Suite 400, 11150 Santa Monica Boulevard, Los Angeles, CA 90025 (US).

(54) Title: A METHOD FOR INHIBITING IMMUNOGLOBULIN-INDUCED TOXICITY RESULTING FROM THE USE OF IMMUNOGLOBULINS IN THERAPY AND IN VIVO DIAGNOSIS



Pleams clearance in high LeY expressing dogs chimeric versus constant region mutant of cBR96-2

(57) Abstract

The present invention provides a method for inhibiting immunoglobulin-induced toxicity resulting from immunotherapy in a subject comprising administering an immunoglobulin or Ig fusion protein molecule to the subject, the immunoglobulin molecule having a variable region and a constant region, the immunoglobulin molecule being modified prior to administration by inactivation of at least a portion of the constant region.

FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AL	Albania	ES	Spain	LS	Lesotho	SI	Slovenia
AM	Armenia	FI	Finland	LT	Lithuania	SK	Slovakia
ΑT	Austria	FR	France	LU	Lixembourg	SN -	Senegal
AU	Australia	GA	Gabon	LV	Latvia	SZ	Swaziland
AZ	Azerbaijan	GB	United Kingdom	MC	Monaco	TD	Chad
BA	Bosnia and Herzegovina	GE	Georgia	MD	Republic of Moldova	TG	Togo
BB	Barbados	GH	Ghana	MG	Madagascar	TJ	Tajikistan
BE	Belgium	GN	Guinea	MK	The former Yugoslav	TM	Turkmenistan
BF	Burkina Faso	GR	Greece		Republic of Macedonia	TR	Turkey
BG	Bulgaria	HU	Hungary	ML	Mali	TT ·	Trinidad and Tobago
ВЈ	Benin	ΙE	Ireland	MN	Mongolia	UA	Ukraine
BR	Brazil	IL	Israel	MR	Mauritania	UG	Uganda
BY	Belarus	IS	Iceland	MW	Malawi	US	United States of America
CA	Canada	IT.	Italy	MX	Mexico	UZ	Uzbekistan
CF	Central African Republic	JP	Japan	NB	Niger	VN	Viet Nam
CG	Congo	KE	Kenya	NL	Netherlands	YU	Yugoslavia
CH	Switzerland	KG	Kyrgyzstan	NO	Norway	zw	Zimbabwe
CI	Cite d'Ivoire	KP	Democratic People's	NZ	New Zealand		
CM	Cameroon		Republic of Korea	PI.	Poland		
CN	China	KR	Republic of Korea	PT	Portugal		
CU	Cuba	KZ	Kazakstan	RO	Romania		
CZ	Czech Republic	LC	Saint Lucia	RU	Russian Federation		
DE	Germany	u	Liechtenstein	SD	Sudan		
DK	Denmark	LK	Sri Lanka	SE	Sweden		
EE	Estonia	LR	Liberia	SG	Singapore		•

5 A METHOD FOR INHIBITING IMMUNOGLOBULIN-INDUCED TOXICITY RESULTING FROM THE USE OF IMMUNOGLOBULINS IN THERAPY AND IN VIVO DIAGNOSIS

Throughout this application various publications are referenced. The disclosures of these publications in their entireties are hereby incorporated by reference into this application in order to more fully describe the state of the art to which this invention pertains.

15 TECHNICAL FIELD OF THE INVENTION

The present invention relates to methods for inhibiting or reducing immunoglobulin-induced toxicity resulting from therapy or in vivo diagnosis. Specifically, in lieu of using unmodified antibodies or recombinant binding proteins for in vivo use, the invention provides the use of modified antibodies or recombinant binding proteins which have been structurally altered in the constant domain so that upon administration immunoglobulin-induced toxicity is reduced or inhibited.

BACKGROUND OF THE INVENTION

25

Over the years investigators have attempted to harness the immune system for therapeutic use. Immunoglobulin (Ig) molecules which constitute an important part of the immune system are of great interest because they (1) react with a diverse family of ligands, (2) possess different effector functions and (3) are of great biological importance. Despite its potential, a persistent problem with

immunoglobulin immunotherapy has been, among other problems, the toxic effect to normal cells of using antibodies which recognize both normal and diseased cells. This problem is far-reaching because the majority of antibodies presently available recognize a target located on both normal and diseased cells (Slavin-Chiorini, et al., Int. J. Cancer 53: 97-103 (1993)).

The constant region can promote cell death through antibody dependent cell mediated cytotoxicity (ADCC) or by complement dependent cytotoxicity (CDC). Despite the deletion of portions of the constant region, particularly the CH₂ domain, the antigen binding function can be retained (D. Yelton, M. Scharf, Mutant monoclonal antibody with alterations in biological functions, J. Exp. Methods 156:1131-1148 (1982)).

10

Others have generated a CH₂-deleted antibody (Mueller et al., Proc. Natl. Acad. Sci. USA 87: 5702-5705 (1990)). Their findings provide that the CH₂-deleted antibody was cleared from the blood of tumor-bearing mice much faster than the corresponding intact antibody. Other in vivo findings also confirmed that a CH₂-deleted antibody, designated ch14.18DCH2, is a potentially useful reagent for radioimmunodetection of human tumors because of its reduced immunogenicity, increased target specificity, and rapid clearance from circulation (Mueller et al., Proc. Natl. Acad. Sci. USA 87: 5702-5705 (1990).

Generally, whole antibody molecules are composed of two heavy (H) and two light (L) chains which are held together by covalent bonds (disulfide) and non-covalent interactions. Each chain contains a variable region (V) and a constant region (C). The variable regions at the amino termini of the two chains form the antigen binding region. The constant region of the H chain has three components or domains. Occasionally, the first constant region domain (CH₁) interacts with the C region of the L chain through hydrophobic interactions and generally a disulfide bond,

depending on isotype. The next C region stretch is the hinge-acting disulfide bond stably introduced between two H chains. The second constant region domain (CH₂) is adjacent to the hinge region. CH₂ contains sequences important for effector functions of the antibody, such as the sequences responsible for complement fixation, and Fc receptor binding The third constant region domain (CH₃) is located at the carboxyl terminus of the H chain, and is considered to play an important role in H chain assembly as well as some C region functions.

Today many antibodies in clinical trials are directed against tumor associated antigens. Most tumor associated antigens are not tumor specific but are also generally found on the cell surface of some normal, non-tumorigenic cells. The clinical use of some antibodies directed against tumor associated antigens are limited because of the toxicity associated with their use. Therefore, there is a need for methods for inhibiting toxicity associated with immunoglobulin use in the field of disease therapy (e.g., therapy for tumors, kidney disease, and the like) and in vivo diagnosis.

We addressed this need by discovering methods for inhibiting or reducing toxicity to normal cells generally associated with immunoglobulin immunotherapy or in vivo diagnosis, wherein the immunoglobulin recognizes both diseased and normal cells. Our discovery involves generating immunoglobulin molecules or Ig fusion proteins having structurally altered constant regions which inhibit or reduce immunoglobulin-induced toxicity.

25 SUMMARY OF THE INVENTION

10

15

20

The present invention provides methods for inhibiting immunoglobulin-induced toxicity by using known immunoglobulin or Ig fusion protein molecules which are structurally altered in their constant regions so that the resulting structurally altered

immunoglobulin or Ig fusion protein molecules exhibit reduced or inhibited toxicity in vivo compared to their original unmodified counterparts.

Structural alteration of the constant region may be effected in a number of ways as long as it results in reducing or inhibiting immunoglobulin-induced toxicity.

In accordance with the practice of the invention, structural alteration of the constant region is effected by deletion of the entire constant region. In another embodiment, only the CH₂ domain is deleted. In another embodiment, only that portion of the CH₂ domain that binds the Fc receptor is deleted. In yet another embodiment, only that portion of the CH₂ domain that binds the complement component Clq is deleted. Alternatively, in another embodiment, multiple deletions in discrete Fc receptor and complement component binding domains are effected.

Alternatively, structural alteration is effected by single or multiple mutations in the CH₂ domain such as amino acid insertions and substitutions. The mutation or mutations must result in inhibiting immunoglobulin-induced toxicity. By way of example, the amino acids in multiple toxicity associated domains in the constant region can be altered so as to render the constant region unable to mediate a ADCC response or activate complement thereby inhibiting immunoglobulin induced toxicity resulting from immunotherapy. Alternatively, multiple amino acids in a single toxicity associated domain in the constant region can be altered.

Further alternatively, structural alteration can be effected by isotype switching resulting in an altered immunoglobulin molecule that either does not induce toxicity or induces some limited toxicity but does not cause a harmful effect. For example, isotype switching can result in the constant region being unable to mediate a CDC or ADCC response or some other activity which mediates toxicity.

5

15

25

BRIEF DESCRIPTION OF THE FIGURES

Figure 1 is a line graph showing plasma clearance in high Le^Y expressing dogs using chimeric BR96 versus constant region mutant of cBR96-2.

Figure 2 is a schematic diagram of a plasmid designated pTWD-cJVK.L1 including the chimeric (c)BR96-light chain (SEQ ID NO. 11).

Figure 3 is a schematic diagram of a plasmid designated pD16hJ1.L1 including the human (h)BR96-light chain (SEQ ID NO. 13).

Figure 4 is a schematic diagram of a plasmid, designated pD17-hJm14-dCH2.H1, of hBR96-2A (i.e., human mutant BR96 having the H1, H2, and H3 mutations and the CH₂ deletion (PCT Application No. 95/305444, published March 6, 1996)).

Figure 5 is a schematic diagram of a plasmid, designated pD17-cJ-dCH2.H1, of cBR96-A (SEQ ID NO. 10) (i.e., chimeric BR96 having the CH₂ deletion (PCT Application No. 95/305444, published March 6, 1996)).

20 Figure 6 is a schematic diagram of a plasmid, designated pD17-cJ.H1, of cBR96.

Figure 7 is a line graph showing the results of an ELISA assay of (1) hBR96-2A-Dox to Le^y (closed diamond), (2) hBR96-2A to Le^y (96:0006A2 R/A)(closed square), (3) hBR96-2A to Le^y (96:0006B R/A)(closed triangle), and BR96-Dox to Le^y (X).

Figure 8 is a line graph showing the results of an ELISA assay of (1) BR96-A-Dox to Le^y (closed diamond), (2) chiBR96 to Le^y (closed square), (3) cBR96-A to Le^y (96:0003 R/A)(closed triangle), and cBR96-Dox to Le^y (X).

Figures 9a-c are schematic diagrams showing the steps for deleting a CH₂ domain.

Figures 10a-c are schematic diagrams showing the construction of BR96 IgG1 CH₂ domain point mutations.

Figure 11 is a schematic diagram showing the construction of the pNg1/14 vector.

Figure 12 is a schematic diagram showing the construction of pD17-hBR96-2.

10

Figure 13 is a schematic diagram showing the construction of pD17-hJm14-dCH2.H1.

Figure 14 is the nucleic acid sequence of pD17-cJ-dCH2.H1, the plasmid shown in Figure 5, chimeric BR96 having the CH₂ deletion.

Figure 15 is a line graph showing the results of an ELISA assay comparing whole chiBR96 and deleted CH₂ chiBR96 on Le^y.

20 Figure 16 is a description of the seven structural alterations.

Figure 17 is a schematic diagram of a plasmid designated pD17-hG1b.

Figure 18 is the nucleic acid sequence of pD17-hJm14.H1.

25

Figure 19 is the nucleic acid sequence of pD17-hG1b.

Figure 20 is a line graph showing complement dependent cytotoxicity. In the legend, the closed square is hBR96-1; closed diamond is hBR96-2B; closed circle is

hBR96-2C; closed triangle is hBR96-2D; open square is hBR96-2H; open circle is hBR96-2A and open triangle is 2B8, anti-*Pseudonomas aeruginosa* flagella type b mAb, negative control.

Figure 21 is a line graph showing antibody dependent cell-mediated cytotoxity. In the legend, the closed square is hBR96-1; closed diamond is hBR96-2B; closed circle is hBR96-2C; closed triangle is hBR96-2D; open square is hBR96-2H; open circle is hBR96-2A and open triangle is 2B8, anti-Pseudonomas aeruginosa flagella type b monoclonal antibody (mAb), negative control.

10

Figure 22 is a line graph showing binding activity of hBR96-2 constant region mutants on LeY-HSA. In the legend, the solid diamond is hBR96-1; solid square is hBR96-2A (CH2 deletion); solid triangle is hBR96-2B (235, 237 mutations); open square is hBR96-2C (318, 320, 322 mutations); open circle is hBR96-2D (331 mutation); and open triangle is hBR96-2H (235, 237, 318, 320, 322, 331 mutations).

20

Figure 23 is a line graph showing binding activity of hBR96-2 constant region mutants on LNFPIII-BSA. LNFPIII is a lacto-N-fucopentasose, a Lewis X trisaccharide with an additional lactose spacer (V Labs, Covington, LA). In the legend, the solid diamond is hBR96-1; solid square is hBR96-2A (CH2 deletion); solid triangle is hBR96-2B (235, 237 mutations); open square is hBR96-2C (318, 320, 322 mutations); open circle is hBR96-2D (331 mutation); and open triangle is hBR96-2H (235, 237, 318, 320, 322, 331 mutations).

25

Figures 24A and 24B provide a strategy for introducing multiple mutations by RPCR. (A) Diagram of he 1.4 kpb IgG heavy chain region showing the hinge CH₂ and CH₃ domains as boxed regions. Site-specific mutations to be introduced into CH₂ positions L1, L2, and L3 are encoded by complementary sets of mutant PCR

primers (A1 and A2; B1 and B2; and C1 and C2). The asterisks (*) indicate the number of amino acid changes introduced at each L position. The two PCR primers, Rs (Recombination -sense) and Ra (Recombination-antisense), flank the Eco-47-III restriction sites and mediate homologous recombination with vector ends. The 3' ends of the oligonucleotides are represented by arrowheads. (B) A three-way homologous recombination event between fragments RsA2, A1Ra and the linearized vector produces the L1 mutant IgG. Two distally located sets of mutations (L1 and L2) are simultaneously introduced by increasing the number of recombining PCR produces as is shown in the four-way recombination of RsA2, A1B1, B1Ra with vector.

10

Figure 25 is a gel showing Eco-47-III restriction endonuclease analysis of DNAs prepared from colonies generated by multiple PCR fragment RPCR. Lane M: 1kb ladder DNA marker (GIBCO/BRL Life Science Technology). Lanes 1-12: Twelve randomly selected colonies resulting from quadruple homologous recombination events were used to prepare plasmid and digested with Eco47-III. Clones 1, 2, 6 and 9 contain the fully assembled 1.4 kpb insert.

Figure 26 provides the amino acid sequence for hBR96-2 heavy-chain variable region and the human IgG1 constant region.

Figure 27 provides the amino acid sequence for hBR96-2A heavy-chain variable region and the human IgG1 constant region.

Figure 28 provides the amino acid sequence for chi BR96 heavy-chain variable region and the human IgG1 constant region without the CH₂ domain.

DETAILED DESCRIPTION OF THE INVENTION

DEFINITIONS

As used herein the term "inhibiting immunoglobulin-induced toxicity" means to reduce or alleviate symptoms generally associated with toxicity caused by immunoglobulin or Ig fusion protein therapy, e.g., toxicity mediated by effector functions of the Fc receptor. For example, BR96 antibody recognizes and binds BR96 antigen which is found at some levels in the gastrointestinal tract and at elevated levels in tumors (as compared to the gastrointestinal tract of normal tissues). The binding of BR96 antibody to BR96 antigen in vivo causes symptoms associated with gastrointestinal toxicity. These symptoms include rapid onset of vomiting, often with blood, and nausea. In humans the bleeding is limited to the fundus of the stomach, causing erosion of the superficial mucosa of the stomach.

15

The pathology of the wound is limited and resolves. However, the extreme nature of the nausea and vomiting, unrelieved by anti-emetics, defines it as the dose-limiting toxicity. For highly elevated levels of other antigens found in the central nervous system (CNS), liver, and other locations, the toxicity will be characterized by symptoms other than those described above.

20

As used herein the term "immunoglobulin molecule" can be produced by B cells or be generated through recombinant engineering or chemical synthetic means. Examples of immunoglobulin molecules include (1) antibodies, e.g., polyclonal and monoclonal antibodies, chimeric or humanized, and (2) recombinant Ig containing binding proteins, e.g., Ig fusion proteins. Recombinant Ig containing binding proteins include cell surface proteins, e.g., CD antigens (in one embodiment, CTLA4), to which an Ig tail is joined.

As used herein the terms "structurally altered" or "structural alteration" means manipulating the constant region so that the resulting molecule or protein exhibits a diminished ability to induce toxicity. Structural alteration can be by chemical modification, proteolytic alteration, or by recombinant genetic means. Recombinant genetic means may include, but is not limited to, the deletion, insertion and substitution of amino acid moieties.

5

10

15

As used herein the terms "multiple toxicity associated domains" means more than one discrete toxicity associated domain. As there appear to be at least two toxicity associated domains in the immunoglobulin molecule, one roughly localized to amino acids 231-238 and another roughly localized to amino acids 310-331, an example of the structural alteration of multiple toxicity associated domains comprises the insertion, substitution or deletion of amino acid residues in both of these domains. This definition excludes structural alterations targeting a single toxicity associated domain.

Merely by way of example, the constant region of the immunoglobulin molecule can be structurally altered so that the molecule no longer mediates a CDC or ADCC response. However, the methods of the invention encompasses the use of structurally altered immunoglobulin molecules regardless of whether it mediates a CDC or ADCC response. The underlying requirement is that the altered molecule must inhibit immunoglobulin-induced toxicity.

Structural alteration can be effected in a number of ways. For example, structural alteration can be effected by deletion of the entire constant region.

Alternatively, structural alteration can be effected by deletion of the entire CH₂ domain of the constant region. In this instance, deletion of the entire CH₂ domain may render the molecule unable to (1) bind an Fc receptor thereby eliminating the

molecule's possibility of mediating antibody-dependent cellular cytotoxicity (ADCC), (2) bind C1q, or (3) activate complement.

Alternatively, structural alteration can be effected by deletion of only that portion of the CH₂ domain that binds the Fc receptor or complement.

Further alternatively, a single mutation or multiple mutations such as substitutions and insertions in the CH₂ domain can be made. The underlying requirement of any mutation is that it must inhibit, diminish, or block immunoglobulin-induced toxicity. For example, this can be achieved by mutating the constant region such that the altered molecule is rendered unable to mediate a CDC response or an ADCC response, or to activate complement.

10

Alternatively, structural alteration can be effected by isotype switching (also known as class switching) so that the altered molecule does not induce toxicity in the subject. In one embodiment, the constant region of the immunoglobulin is structurally altered so that it no longer binds the Fc receptor or a complement component, e.g., switching a molecule's original IgG isotype from IgG1 to IgG4. Isotype switching can be effected regardless of species, i.e., an isotype from a non-human being can be switched with an isotype from a human being (E.D. Finkelman et al. (1990) Annu. Rev. Immunol. 8:303-333; T. Honjo et al. (1979) Cell 18: 559-568; T. Honjo et al. In "Immunoglobulin Genes" pp. 124-149 Academic Press, London)).

As used herein the term "Ig fusion protein" means any recombinantly produced antigen or ligand binding domain having a constant region which can be structurally altered.

As used herein "cytotoxic agent" includes antimetabolites, alkylating agents, anthracyclines, antibiotics, anti-mitotic agents, and chemotherapeutic agents. Specific examples within these groups include but are not limited to ricin, doxorubicin, daunorubicin, taxol, ethidium bromide, mitomycin, etoposide, tenoposide, vincristine, vinblastine, colchicine, supporin, gelonin, PE40, bryodin, dihydroxy anthracin dione, actinomycin D, and 1-dehydrotestosterone.

As used herein the term "BR96" refers to (1) the whole BR96 monoclonal antibody disclosed in PCT No. 95/305444, published March 6, 1996, (2) chimeric BR96 monoclonal antibody disclosed in PCT No. 95/305444, published March 6, 1996, or (3) BR96 mutant molecules disclosed in PCT No. 95/305444, published March 6, 1996.

10

25

As used herein, "treating" means to (1) provide tumor regression so that the tumor is not palpable for a period of time (standard tumor measurement procedures may be followed (A.B. Miller et al. "Reporting results of cancer treatment" Cancer 47:207-214 (1981)); (2) stabilize the disease; or (3) provide any clinically beneficial effects.

As used herein, an "effective amount" is an amount of the antibody, 20 immunoconjugate, or recombinant molecule which kills cells or inhibits the proliferation thereof.

As used herein, "administering" means oral administration, administration as a suppository, topical contact, intravenous, intraperitoneal, intramuscular or subcutaneous administration, or the implantation of a slow-release device such as a miniosmotic pump, to the subject.

As used herein, "pharmaceutically acceptable carrier" includes any material which when combined with the antibody retains the antibody's specificity or efficacy and is

non-reactive with the subject's immune system. Examples include, but are not limited to, any of the standard pharmaceutical carriers such as a phosphate buffered saline solution, water, emulsions such as oil/water emulsion, and various types of wetting agents. Other carriers may also include sterile solutions, tablets including coated tablets and capsules.

Typically such carriers contain excipients such as starch, milk, sugar, certain types of clay, gelatin, stearic acid or salts thereof, magnesium or calcium stearate, talc, vegetable fats or oils, gums, glycols, or other known excipients. Such carriers may also include flavor and color additives or other ingredients. Compositions comprising such carriers are formulated by well known conventional methods.

As used herein, "mutation" means a single amino acid or nucleic acid mutation or multiple mutations by whatever means, e.g., homologous recombination, error prone PCR, or site directed mutagenesis.

In order that the invention herein described may be more fully understood, the following description is set forth.

20 METHODS OF THE PRESENT INVENTION

10

25

The present invention provides a method for inhibiting immunoglobulin-induced toxicity resulting from the use of immunoglobulin during therapy or in vivo diagnosis. For example, the methods of the invention would be useful to minimize the toxicity associated with prolonged clinical exposure to immunoglobulin use during or after tumor imaging with radiolabeled antibodies.

In accordance with the practice of this invention, the subject includes, but is not limited to, human, equine, porcine, bovine, murine, canine, feline, and avian

subjects. Other warm blooded animals are also included in this invention.

This method comprises administering an immunoglobulin molecule to the subject. The immunoglobulin can be IgG, IgM, or IgA. IgG is preferred.

5

In one embodiment of the invention, the immunoglobulin molecule recognizes and binds Le^x. In another embodiment, the immunoglobulin recognizes and binds Le^x. In a further embodiment, the immunoglobulin is a monoclonal antibody BR96 produced by the hybridoma deposited on February 22, 1989 with the American Type Culture Collection (ATCC), 12301 Parklawn Drive, Rockville, MD 20852 and accorded ATCC Accession No.: HB 10036. In yet another embodiment, the immunoglobulin is a chimeric antibody ChiBR96 produced by the hybridoma deposited on May 23, 1990, with the ATCC, 12301 Parklawn Drive, Rockville, MD 20852 and accorded ATCC Accession No.: HB 10460.

15

20

10

In accordance with the practice of the invention, the immunoglobulin can be a bispecific antibody with a binding specificity for two different antigens, one of the antigens being that with which the monoclonal antibody BR96 produced by the hybridoma having the identifying characteristics of HB 10036 as deposited with the ATCC binds. Also, in accordance with the practice of the invention, the immunoglobulin can be an anti-idiotypic antibody.

As required by the invention, at least a portion of the constant region of the immunoglobulin molecule is structurally altered. Structural alteration can be effected by a number of means. In one embodiment, the entire constant region, i.e., CH₁, CH₂, and CH₃ domains, can be deleted.

In another embodiment, only the CH₂ domain is deleted from the immunoglobulin molecule (e.g., cBR96-A (Figure 5), hBR96-2A (Figure 4). In this embodiment, the

CH₂ deletion may result in a molecule unable to bind the Fc receptor or a complement component.

In another embodiment, only that portion of the CH₂ domain which binds the complement component Clq is deleted. In yet another embodiment, mutations in specific portions of the CH₂ domain are made. For example, the immunoglobulin molecule may be modified by structurally altering multiple toxicity associated domains in the constant region so that immunoglobulin-induced toxicity is inhibited. A discussion of such mutations are further found hereinafter.

10

20

25

Regardless of the means, the underlying requirement for any structural alteration of the constant region is that immunoglobulin-induced toxicity is substantially reduced or inhibited. In one embodiment, immunoglobulin-induced toxicity is inhibited by structurally altering the constant region such that the molecule's ability to mediate a CDC response or ADCC response and/or activate the complement cascade is prevented or inhibited. Methods for determining whether the molecule is able to inhibit a CDC response are well known, e.g., one method involves a ⁵¹Cr-release test (H. Garrigues et al. Int. J. Cancer 29:511 (1982); I. Hellström et al. PNAS 82:1499 (1985)). Methods for determining whether the molecule is able to inhibit an ADCC response are well known (I. Hellström et al. PNAS 82:1499 (1985)). Methods for determining whether the molecule is able to activate a complement cascade are well known.

In another embodiment of the invention, the method comprises administering to the subject an Ig fusion protein having a structurally altered constant region. Structural alteration of the constant region may include deletion of the entire C region or portions thereof, e.g., alteration of the CH₂ domain so that the altered molecule no longer binds the Fc receptor or a complement component.

The invention further provides a method for inhibiting immunoglobulin-induced toxicity resulting from immunotherapy in a subject. The method comprises administering to the subject an antibody which has been modified so that at least a portion of the constant region has been structurally altered as discussed supra. In one embodiment, the antibody recognizes and binds Le^x. In another embodiment, the antibody recognizes and binds to Le^x.

In accordance with the practice of this invention, the antibody can be monoclonal antibody BR96 produced by the hybridoma having the identifying characteristics of HB 10036 as deposited with the ATCC. Alternatively, the antibody can be chimeric antibody ChiBR96 produced by the hybridoma having the identifying characteristics of HB 10460 as deposited with the ATCC. Further, the antibody can be a bispecific antibody with a binding specificity for two different antigens, one of the antigens being that with which the monoclonal antibody BR96 produced by the hybridoma having the identifying characteristics of HB 10036 as deposited with the ATCC binds.

Additionally, the present invention provides a method for inhibiting immunoglobulin-induced toxicity resulting from immunotherapy for a disease in a subject. The disease will vary with the antigen sought to be bound. Examples of diseases include but are not limited to immunological diseases, cancer, cardiovascular diseases, neurological diseases, dermatological diseases or kidney disease.

20

This method comprises the following steps. Step one provides selecting an antibody for a target. Generally, the target is associated with the disease and the antibody directed to the target is known. For example, the target can be the BR96 antigen and the antibody selected is BR96.

Step two of this method provides structurally altering the constant region of the antibody so selected so that immunoglobulin induced toxicity is inhibited. Inactivation can include any of the means discussed above. For example, inactivation can be effected by structurally altering multiple toxicity associated domains in the CH₂ domain of the constant region of the lg protein so selected.

Step three of this method provides administering the structurally altered antibody of step two to the subject under conditions that the structurally altered antibody recognizes and binds the target and that such binding directly or indirectly alleviates symptoms associated with the disease.

In accordance with the invention, in one embodiment step one provides selecting an Ig fusion protein for a target. Further, the method provides mutating the Ig fusion protein so selected by structurally altering the CH₂ domain of the constant region of the Ig protein by the same means discussed above.

15

20

The invention further provides methods to treat human carcinoma. For example, the immunoglobulin, antibody, or Ig fusion protein discussed above can be used in combination with standard or conventional treatment methods such as chemotherapy, radiation therapy or can be conjugated or linked to a therapeutic drug, or toxin, as well as to a lymphokine or a tumor-inhibitory growth factor, for delivery of the therapeutic agent to the site of the carcinoma.

Techniques for conjugating therapeutic agents to immunoglobulins are well known (see, e.g., Arnon et al., "Monoclonal Antibodies For Immunotargeting Of Drugs In Cancer Therapy", in Monoclonal Antibodies And Cancer Therapy, Reisfeld et al. (eds.), pp. 243-56 (Alan R. Liss, Inc. 1985); Hellström et al., "Antibodies For Drug Delivery", in Controlled Drug Delivery (2nd Ed.), Robinson et al. (eds.), pp. 623-53 (Marcel Dekker, Inc. 1987); Thorpe, "Antibody Carriers Of Cytotoxic Agents In

Cancer Therapy: A Review", in Monoclonal Antibodies '84: Biological And Clinical Applications, Pinchera et al. (eds.), pp. 475-506 (1985); and Thorpe et al., "The Preparation And Cytotoxic Properties Of Antibody-Toxin Conjugates", Immunol. Rev., 62:119-58 (1982)).

5

Alternatively, the structurally altered antibody or lg fusion protein can be coupled to high-energy radiative agents, e.g., a radioisotope such as ¹³¹I; which, when localized at the tumor site, results in a killing of several cell diameters (see, e.g., Order, "Analysis, Results, And Future Prospective Of The Therapeutic Use Of Radiolabeled Antibody In Cancer Therapy", in Monoclonal Antibodies For Cancer Detection And Therapy, Baldwin et al. (eds.), pp. 303-16 (Academic Press 1985)). According to yet another embodiment, the structurally altered BR96 antibody can be conjugated to a second antibody to form an antibody heteroconjugate for the treatment of tumor cells as described by Segal in United States Patent 4,676,980.

15

20

Still other therapeutic applications for the structurally altered antibody or Ig fusion protein of the invention include conjugation or linkage, e.g., by recombinant DNA techniques or protein chemical techniques, to an enzyme capable of converting a prodrug into a cytotoxic drug and the use of that antibody-enzyme conjugate in combination with the prodrug to convert the prodrug to a cytotoxic agent at the tumor site (see, e.g., Senter et al., "Anti-Tumor Effects Of Antibody-alkaline Phosphatase", Proc. Natl. Acad. Sci. USA, 85:4842-46 (1988); "Enhancement of the in vitro and in vivo Antitumor Activities of Phosphorylated Mitomycin C and Etoposide Derivatives by Monoclonal Antibody-Alkaline Phosphatase Conjugates", Cancer Research 49:5789-5792 (1989); and Senter, "Activation of Prodrugs by Antibody-Enzyme Conjugates: A New Approach to Cancer Therapy," FASEB J. 4:188-193 (1990)).

It is apparent therefore that the present invention encompasses pharmaceutical compositions including immunoglobulin molecules, antibodies, and Ig fusion proteins all having structurally altered CH₂ domains, and their use in methods for treating human carcinomas. For example, the invention includes pharmaceutical compositions for use in the treatment of human carcinomas comprising a pharmaceutically effective amount of a structurally altered BR96 and a pharmaceutically acceptable carrier.

The compositions may contain the structurally altered antibody or Ig fusion protein or antibody fragments, either unmodified, conjugated to a therapeutic agent (e.g., drug, toxin, enzyme or second antibody). The compositions may additionally include other antibodies or conjugates for treating carcinomas (e.g., an antibody cocktail).

The compositions of the invention can be administered using conventional modes of administration including, but not limited to, intrathecal, intravenous, intraperitoneal, oral, intralymphatic or administration directly into the tumor. Intravenous administration is preferred.

The composition of the invention can be in a variety of dosage forms which include,

but are not limited to, liquid solutions or suspensions, tablets, pills, powders,
suppositories, polymeric microcapsules or microvesicles, liposomes, and injectable
or infusible solutions. The preferred form depends upon the mode of administration
and the therapeutic application.

The compositions of the invention also preferably include conventional pharmaceutically acceptable carriers and adjuvants known in the art such as human serum albumin, ion exchangers, alumina, lecithin, buffer substances such as phosphates, glycine, sorbic acid, potassium sorbate, and salts or electrolytes such as protamine sulfate.

In accordance with the practice of the invention, the pharmaceutical carrier can be a lipid carrier. The lipid carrier can be a phospholipid. Further, the lipid carrier can be a fatty acid. Also, the lipid carrier can be a detergent. As used herein, a detergent is any substance that alters the surface tension of a liquid, generally lowering it.

In one example of the invention, the detergent can be a nonionic detergent. Examples of nonionic detergents include, but are not limited to, polysorbate 80 (also known as Tween 80 or (polyoxyethylenesorbitan monooleate), Brij, and Triton (for example Triton WR-1339 and Triton A-20).

Alternatively, the detergent can be an ionic detergent. An example of an ionic detergent includes, but is not limited to, alkyltrimethylammonium bromide.

Additionally, in accordance with the invention, the lipid carrier can be a liposome.

As used in this application, a "liposome" is any membrane bound vesicle which contains any molecules of the invention or combinations thereof.

The most effective mode of administration and dosage regimen for the compositions of this invention depends upon the severity and course of the disease, the patient's health and response to treatment and the judgment of the treating physician.

The interrelationship of dosages for animals of various sizes and species and humans based on mg/m² of surface area is described by Freireich, E.J., et al. Cancer Chemother., Rep. 50 (4): 219-244 (1966). Adjustments in the dosage regimen can be made to optimize the tumor cell growth inhibiting and killing response, e.g., doses can be divided and administered on a daily basis or the dose reduced proportionally depending upon the situation (e.g., several divided doses can be

administered daily or proportionally reduced depending on the specific therapeutic situation).

THE MOLECULES OF THE INVENTION

5

10

The present invention provides structurally altered BR96 or BR96 Ig fusion proteins. Structurally altered BR96 antibodies or Ig fusion proteins have the variable region of BR96 and a modified constant region. This modification provides structurally altered BR96 antibodies or Ig fusion proteins with the ability to inhibit immunoglobulin-induced toxicity.

Various embodiments of structurally altered BR96 or BR96 Ig fusion proteins have been made.

- In one embodiment, designated cBR96-A, the entire CH₂ domain of cBR96 was deleted. CBR96-A is expressed by the plasmid having the sequence shown in SEQ. ID. NO. 10. cBR96 is expressed by a plasmid having the sequence in SEQ ID NO. 9.
- In another embodiment, designated hBR96-2A, the entire CH₂ domain of hBR96 was deleted. hBR96-A is expressed by the plasmid having the sequence shown in SEQ. ID. NO. 12. hBR96 is a mutant BR96 having the H1, H2, and H3 mutations described in PCT Application No. 95/305444, published March 6, 1996.
- In yet another embodiment, designated hBR96-2B, the leucine residue located at amino acid position 235 is mutated to alanine. Additionally, the glycine residue located at amino acid position 237 is mutated to alanine. The amino acid position numbering used is described in Kabat et al. Sequences of Proteins of Immunological Interest 5th Edition (1991) United States Department of Health and Human Services.

In a further embodiment, designated hBR96-2C, the glutamic acid residue at position 318 is mutated to serine; the lysine residue located at position 320 is mutated to serine; and the lysine residue located at position 322 is mutated to serine using standard protocols (Alexander R. Duncan and Greg Winter "The binding site for C1q on IgG" Nature 332:738 (1988)).

In another embodiment, designated hBR96-2D, the proline residue at position 331 is mutated to alanine (M-H. Tao et al., "Structural features of human immunoglobulin G that determine isotype-specific differences in complement activation" J. Exp. Med. 178:661-667 (1993); Y. Xu et al., "Residue at position 331 in the IgG1 and IgG4 domains contributes to their differential ability to bind and activate complement" J. Biol. Chem. 269:3469-3474 (1994)).

In an additional embodiment, designated hBR96-2E, the leucine residue at position 235 is mutated to alanine; the glycine residue located at position 237 is mutated to alanine; the glutamic acid residue located at position 318 is mutated to serine; the lysine residue located at position 320 is mutated to serine; and the lysine residue located at position 322 is mutated to serine (A. Morgan et al., "The N-terminal end of the CH₂ domain of chimeric human IgG1 anti-HLA-DR is necessary for C1q, Fc(gamma)RI and Fc(gamma)RIII binding" Immunol. 86:319-324 (1995)).

In yet a further embodiment, designated hBR96-2F, the leucine residue located at position 235 is mutated to alanine; the glycine residue located at position 237 is mutated to alanine; and the proline residue located at position 331 is mutated to alanine.

In yet another embodiment, designated hBR96-2G, the glutamic acid residue located at position 318 is mutated to serine; the lysine residue located at position 320 is

mutated to serine; the lysine residue located at position 322 is mutated to serine; and the proline residue located at position 331 is mutated to alanine.

In another embodiment, designated hBR96-2H, the leucine residue located at position 235 is mutated to alanine; the glycine residue located at position 237 is mutated to alanine; the glutamic acid residue at position 318 is mutated to serine; the lysine residue located at position 320 is mutated to serine; the lysine residue located at position 322 is mutated to serine; and the proline residue located at position 331 is mutated to alanine.

10

15

20

25

Depending on its form, a structurally altered BR96 antibody or fusion protein can be a monofunctional antibody, such as a monoclonal antibody, or bifunctional antibody, such as a bispecific antibody or a heteroantibody. The uses of structurally altered BR96, i.e., as a therapeutic or diagnostic agent, will determine the different forms of structurally altered BR96 which is made.

Several options exists for antibody expression. Immunoexpression libraries can be combined with transfectoma technology, i.e., the genes for the Fab molecules derived from the immunoglobulin gene expression library can be connected to the desired constant-domain exons. These recombinant genes can then be transfected and expressed in a transfectoma that would secrete an antibody molecule.

Once produced, the polypeptides of the invention can be modified, i.e., by amino acid modifications within the molecule, so as to produce derivative molecules. Such derivative molecules would retain the functional property of the polypeptide, namely, the molecule having such substitutions will still permit the binding of the polypeptide to the BR96 antigen or portions thereof.

It is a well-established principle of protein chemistry that certain amino acid

substitutions, entitled "conservative amino acid substitutions," can frequently be made in a protein without altering either the conformation or the function of the protein.

5 Amino acid substitutions include, but are not necessarily limited to, amino acid substitutions known in the art as "conservative".

Such changes include substituting any of isoleucine (I), valine (V), and leucine (L) for any other of these hydrophobic amino acids; aspartic acid (D) for glutamic acid (E) and vice versa; glutamine (Q) for asparagine (N) and vice versa; and serine (S) for threonine (T) and vice versa.

Other substitutions can also be considered conservative, depending on the environment of the particular amino acid and its role in the three-dimensional structure of the protein. For example, glycine (G) and alanine (A) can frequently be interchangeable, as can alanine and valine (V).

15

20

Methionine (M), which is relatively hydrophobic, can frequently be interchanged with leucine and isoleucine, and sometimes with valine. Lysine (K) and arginine (R) are frequently interchangeable in locations in which the significant feature of the amino acid residue is its charge and the differing pK's of these two amino acid residues are not significant. Still other changes can be considered "conservative" in particular environments.

In one embodiment of the present invention, the polypeptide is substantially pure, i.e., free of other amino acid residues which would inhibit or diminish binding of the polypeptide to its target and would inhibit or reduce gastrointestinal toxicity which are normally exhibited during or after antibody therapy.

NUCLEIC ACID MOLECULES ENCODING THE PRESENT INVENTION

The nucleotide sequences and the amino acid sequences of the variable and constant regions of BR96 are known. The sequence for the immunoglobulin constant region is known and provided in Figure 18. Specific mutations in the constant region of the BR96 antibody were made. Nucleic acid molecules encoding the seven mutants described above (hBR96-2B through hBR96-2H) are as follows.

In hBR96-2B, alanine at amino acid positions 235 and 237 is encoded by codons 0 GCU, GCC, GCA, or GCG.

In hBR96-2C, serine at positions 318, 320, and 322 is encoded by UCU, UCC, UCA, or UGG.

15 In hBR96-2D, alanine at position 331 is encoded by codons GCU, GCC, GCA, or GCG.

In hBR96-2E, alanine at positions 235 and 237 is encoded by codons GCU, GCC, GCA, or GCG. Serine at positions 318, 320, and 322 is encoded by UCU, UCC, UCA, or UGG.

20

In hBR96-2F, alanine at positions 235, 237, and 331 is encoded by codons GCU, GCC, GCA, or GCG.

In hBR96-2G, serine at positions 318, 320, 322 is encoded by UCU, UCC, UCA, or UGG. Further, the alanine at position 331 is encoded by codons GCU, GCC, GCA, or GCG.

In hBR96-2H, alanine at positions 235, 237, and 331 is encoded by codons GCU,

GCC, GCA, or GCG. Additionally, serine at positions 318, 320, 322 is encoded by UCU, UCC, UCA, or UGG.

Any of the above can be deoxyribonucleic acid (DNA), e.g., complementary DNA (cDNA), or ribonucleic acid (RNA).

IMMUNOCONJUGATES

Immunoconjugates (having whole antibody or Ig fusion proteins) may be constructed using a wide variety of chemotherapeutic agents such as folic acid and anthracyclines (Peterson et al., "Transport And Storage Of Anthracyclines In Experimental Systems And Human Leukemia", in Anthracycline Antibiotics In Cancer Therapy, Muggia et al. (Eds.), p. 132 (Martinus Nijhoff Publishers (1982); Smyth et al., "Specific Targeting of Chlorambucil to Tumors With the Use of Monoclonal Antibodies", J. Natl. Cancer Inst., 76:503-510 (1986)), including doxorubicin (DOX) (Yang and Reisfeld "Doxorubicin Conjugated with a Monoclonal Antibody Directed to a Human Melanoma-Associated Proteoglycan Suppresses Growth of Established Tumor xenografts in Nude Mice PNAS (USA)" 85:1189-1193 (1988)), Daunomycin (Amon and Sela "In Vitro and in vivo Efficacy of Conjugates of Daunomycin With Anti-Tumor Antibodies" Immunol. Rev., 65:5-20 27 (1982)), and morpholinodoxorubicin (Mueller et al., "Antibody Conjugates With Morpholinodoxorubicin and Acid-Cleavable Linkers", Bioconjugate Chem., 1:325-330 (1990)).

BR96 has been conjugated to doxorubicin and has been shown to be effective in therapy of certain cancers or carcinomas (Trail, P.A., Willner, D., Lasch, S.J., Henderson, A.J., Casazza, A.M., Firestone, R.A., Hellström, I., and Hellström, K.E. Cure of xenografted human carcinomas by BR96-doxorubicin immunoconjugates. Science, 261:212-215, 1993).

In accordance with the practice of the invention, structurally altered BR96 can be used in forms including unreduced IgG, reduced structurally altered IgG, and fusion proteins (PCT Application No. 95/305444, published March 6, 1996).

5

Suitable therapeutic agents for use in making the immunoconjugate includes Pseudomonas exotoxin A (PE) in either the native PE or LysPE40 form. LysPE40 is a truncated form containing a genetically modified amino terminus that includes a lysine residue for conjugation purposes. Doxorubicin is also a suitable therapeutic agent.

Additional examples of therapeutic agents include, but are not limited to, antimetabolites, alkylating agents, anthracyclines, antibiotics, and anti-mitotic agents.

15

10

Antimetabolites include methotrexate, 6-mercaptopurine, 6-thioguanine, cytarabine, 5-fluorouracil decarbazine.

Alkylating agents include mechlorethamine, thiotepa chlorambucil, melphalan,
carmustine (BSNU) and lomustine (CCNU), cyclothosphamide, busulfan,
dibromomannitol, streptozotocin, mitomycin C, and cis-dichlorodiamine platinum
(II) (DDP) cisplatin.

Anthracyclines include daunorubicin (formerly daunomycin) and doxorubicin (also referred to herein as adriamycin). Additional examples include mitozantrone and bisantrene.

Antibiotics include dactinomycin (formerly actinomycin), bleomycin, mithramycin, and anthramycin (AMC).

Antimitotic agents include vincristine and vinblastine (which are commonly referred to as vinca alkaloids).

Other cytotoxic agents include procarbazine, hydroxyurea, asparaginase, corticosteroids, mytotane (O,P'-(DDD)), interferons.

Further examples of cytotoxic agents include, but are not limited to, ricin, bryodin, gelonin, supporin, doxorubicin, taxol, cytochalasin B, gramicidin D, ethidium bromide, etoposide, tenoposide, colchicine, dihydroxy anthracin dione, 1-dehydrotestosterone, and glucocorticoid.

Clearly analogs and homologs of such therapeutic and cytotoxic agents are encompassed by the present invention. For example, the chemotherapuetic agent aminopterin has a correlative improved analog namely methotrexate.

Further, the improved analog of doxorubicin is an Fe-chelate. Also, the improved analog for 1-methylnitrosourea is lomustine. Further, the improved analog of vinblastine is vincristine. Also, the improved analog of mechlorethamine is cyclophosphamide.

METHODS FOR MAKING MOLECULES OF THE INVENTION

20

There are multiple approaches to making site specific mutations in the CH₂ domain of an immunoglobulin molecule. One approach entails PCR amplification of the CH₂ domain with the mutations followed by homologous recombination of the mutated CH₂ into the vector containing the desired immunoglobulin, e.g., hBR96-2. For example, hBR96-2B and hBR96-2D have been made by this method.

Another approach would be to introduce mutations by site-directed mutagenesis of single-stranded DNA. For example, vector pD17-hG1b, which contains only the constant region of IgG1 and not the V domain of hBR96, has the f1 origin of replication. This gives the vector the properties of a phagemid and site-directed mutagenesis experiments can be performed according to the methods of Kunkel, et al. (Kunkel, T.A., J.D. Roberts, and R.A. Zakour, 1987 Methods Enzymol. 154:367-383) as provided in the Bio-Rad Muta-Gene® phagemid *in vitro* mutagenesis kit, version 2. For example, hBR96-2B, -C, -D, -E, -F, -G, and -H were made by this method.

10

In order that the invention described herein may be more fully understood, the following examples are set forth. It should be understood that these examples are for illustrative purposes only and are not to be construed as limiting the scope of this invention in any manner.

15

EXAMPLE 1

The following standard ELISA protocol was used.

20 Materials: Immulon2 96 well plates and Genetic Systems Specimen Diluent Concentrate (10x); antibody conjugate was Goat Anti Human Kappa-HRP Mouse Adsorbed, Southern Biotech. at 1:10,000 in Genetic Systems Conjugate Diluent (1x); Genetic Systems EIA Chromogen Reagent (TMB) (1:100); Genetic Systems EIA Buffered Substrate (1x); primary antibody or antigen were AffiniPure F(ab')₂

Fragment Goat Anti Human IgG Fc Fragment specific (Jackson Immuno Research),
Goat Anti Human Kappa-UNLB (Southern Biotechnology Associates), Le^y-HSA
(Alberta Research Council).

Methods: Dilute primary antibody or antigen to 1.0 μ g/ml in 0.05M Carb/Bicarb buffer. Add 100 μ l of the diluted solution per well in Immulon 2 plates. Seal plates and incubate O.N. at 4°C.

Block plates by flicking them and blotting on paper towels. Add 200μl/well of Genetic Systems, Specimen Diluent Concentrate (1x). Incubate at least 1 hour at room temperature and then dump the contents of the plates. Wash the plates 3x in saline/Tween. Blot to dry. Allow the plates to dry at R.T. (45 min. to 1 hour). Seal and store the plates at 4°C.

10

Test samples as follows. Dilute samples and standards in Specimen Diluent at 1:10. Perform serial dilutions in separate round bottom plates. Transfer 100µl/well of final dilutions to antigen coated assay plates; then incubate O.N. at 4°C. Wash plates 3x with saline/Tween.

15

For conjugation add 100 µl/well of antibody-HRP conjugate in Genetic Systems Conjugate Diluent (1x). Incubate plates at Room Temp. for 60 min. Wash plates 3x in saline/Tween.

20 Add 100 µl/well of Genetic Systems EIA Chromogen Reagent (TMB) 1:100 in EIA Buffered Substrate (1x). Incubate at R.T. for 15 min. and stop with 1N H₂SO₄ 100 µl/well. Read plate at 450/630nm in EIA plate reader.

EXAMPLE 2

25

Construction of CH₂ deleted BR96 molecules

Strategy for Deleting CH₂ Domains: To construct CH₂ deleted BR96 molecules, the hinge, CH₂ and CH₃ domains were removed from chimeric BR96 and humanized

BR9696-2 IgG1 molecules by an Eco47-III restriction digestion in non-coding regions. The hinge and CH₃ domains were amplified by polymerase chain reaction (PCR) from a human IgG1 (pNγ1.14) molecule lacking the CH₂ domain. Two oligonucleotides (Sense 49mer, Antisense 50mer) homologous to the sequences of IgG1 constant region at both sides preserving E.co47-III sites were synthesized. The amplified hinge and CH₃ domain PCR fragments were added into Eco47-III sites on BR96 IgG1 molecules by in vivo homologous recombination (P. Bubeck et al., Nucleic Acid Research (1993) 21:3601-3602). The new BR96 IgG1 molecules were verified by restriction mapping and sequencing.

10

A sewing PCR strategy was used for the construction of CH₂ deleted human IgG1 (pNγ1.14) (Robert M. Horton, et al. (1990) Biotech 8 (5)P, 528).

The CH₁ domain was amplified as a 580 bp fragment with a sense oligonucleotide

(5' TGG CAC CGA AAG CTT TCT GGG GCA GGC CAG GCC TGA 3') (primer
A) and an antisense oligonucleotide (5' TCC GAG CAT GTT GGT ACC CAC

GTG GTG GTC GAC GCT GAG CCT GGC TTC GAG CAG ACA 3') (primer B)

from a linearized human IgG1 constant region vector (pNγ1.7). The PCR fragment
extends from the 5' end of the Hind-III site (in bold) through the Cel-II, Sal-I, Dra
III, Kpn-I, 6 bp nucleotide spacer and Mro-I sites (in bold) at the 3' end of the CH₁
domain.

The CH₃ domain was then partially amplified (to the Xba-I site) with a sense primer (5' GTC GAC CAC GTG GGT ACC AAC ATG TCC GGA GCC ACA

TGG ACA GAG GCC GGC T 3') (primer C) and an antisense primer (5' CTG GTT CTT GTT CAT CTC CTC TCT AGA TGG 3') (primer D) from a linearized human lgG1 constant region vector (pNγ1.7). A PCR fragment (about 150 bp) with Sal-I, Dra-III, Kpn-I, 6 nucleotide spacer and Mro-I sites (in bold) on its 5' end, extends only through the Xba-1 site (in bold) within the CH₃ domain.

The CH₁ and CH₃ partial PCR fragments were combined in a PCR without any primer. The reaction was run through two full cycles of denaturation and reannealing to allow the fragments to combine at the homologous region at the 3' ends. Primers A and D (described above) were added to the reaction and the PCR cycle was completed. The polymerase extends the DNA with primer A and primer D, yielding a full-length (660 bp) PCR fragment. The newly extended PCR fragment is arranged from the 5' end to the 3' end in the following order: Hind-III - CH₁ - Cel-II - Sal-I - Dra-III - Kpn-I - 6 bp spacer - Mro-I - CH₃ partial - Xba-1.

10

The combined PCR fragment, with the CH₁ and partial CH₃ domains, was then cloned by a blunt end ligation into a Sma-I site on a pEMBL18 vector and the sequence was confirmed by dideoxy sequencing (Sanger et al. (1977) PNAS (USA) 74:5463-5466).

15

To transfer the CH_1 and partial CH_3 into a mammalian expression vector, both the pEMBL18 and pN γ 1.7 vectors were digested with Hind-III and Xba-I. The Hind-III and Xba-I fragment was ligated into the same sites on a linearized pN γ 1.7 vector. The new construct, with CH_1 and a full CH_3 domain, was designated the pN γ 1.10 vector.

20

25

The hinge fragment was amplified from a Hind-III digested pNγ1.7 vector with the primers designed to flank the hinge exon with a Sal-I and a Dra-III cloning site at each end. These sites also exist between the CH₁ and CH₃ domains of the pNγ1.10 construct. The sense oligonucleotide (5' ACC ATG GTC GAC CTC AGA CCT GCC AAG AGC CAT ATC 3') with a 6 bp spacer and a Sal-I cloning site (in bold) and the antisense oligonucleotide (5' CAT GGT CAC GTG GTG TGT CCC TGG ATG CAG GCT ACT CTA G 3') with a 6 bp spacer and a Dra-III cloning site (in bold) were used for the amplication of the hinge fragment (250 bp).

The hinge region PCR fragment was cloned into a Sma-I site on pEMBL18 by blunt end ligation. Both the pEMBL18 with the hinge domain and the pN γ 1.10 with the CH₂ and CH₃ domains were digested with Sal-1 and Dra-III. The digested hinge fragment was cloned into the Sal-1 and Dra-III linearized sites on the pN γ 1.10 vector. The new construct, now carrying the CH₁, hinge and CH₃ domains, was designated pN γ 1.11.

To make the final CH₂ deleted human IgG1 construct, both the pNγ1.11 construct and pNγ1.11 vector were digested with BamH1 and HindIII. A fragment containing the CH₁, hinge and CH₃ domains was cloned into the linearized pNγ1.11 vector. The new constant region IgG1 construct lacks the CH₂ domain and is designated pNγ1.14 (Figure 11).

15 For digestion of BR96 IgG1 with Eco47-III, a restriction fragment with hinge, CH₂ and CH₃ domains was identified on the constant region sequence of BR96 IgG1 vector in both chimeric and humanized molecules. The 5' end of this fragment lies inside the intron between CH₁ and hinge and the 3' end is located inside the CH₃ intron of the BR96 IgG1 molecule. The hinge, CH₂ and CH₃ domains (1.368 kb fragment) were removed from BR96 IgG1 molecules by Eco47-III restriction digestion. The Eco47-III is a blunt end cutter. The BR96 IgG1 DNA digested with this enzyme does not require any pretreatment before cloning. Figure 12 is a diagrammatic representation of the pD17-hBR96-2 vector showing the Eco47-III sites used in cloning.

25

The CH_2 deleted BR96 IgG1 was then constructed as follows. The hinge and CH_3 domains were amplified from a CH_2 deleted L6 IgG1 (pN γ 1.14) construct with a sense oligonucleotide (5'

CAGGGAGGGAGGTGTCTGCTGGAAGCCAGGCTCAGCGCTGACCTCAG

A 3') homologous to the constant region sequence of IgG1 at the 5' end of the Eco47-III site (in bold) and an antisense oligonucleotide

(5'GGAAAGAACCATCACAGTCTCGCAGGGG

- CCCAGGGCAGCGCTGGGTGCTT 3') homologous to the constant region sequence of IgG1 at the 3' end of the Eco47-III site (in bold). The Eco47-III site at the 3' end of the pNγ1.14 construct is modified in the cloning process. The Eco47-III site is thus introduced into an antisense primer and used in amplification of the hinge and CH₃ domains.
- The pD17-BR96 IgG1 vector was digested with Eco47-III and the hinge, CH₂ and CH₃ domains were removed. The linearized pD17-BR96 IgG1 vector was mixed with equimolar amounts of hinge and CH₃ PCR fragments. Cotransformation of the PCR fragment with linearized DNA into E.coli DH5a competent cells resulted in a recombinant molecule, mediated by homologous recombination in bacteria. This construct lacks the CH₂ domain of BR96 IgG1 molecules, and is designated pD17-BR96-dCH2 (Figure 13).
 - 1.9 grams of CH₂-deleted chimeric BR96 was obtained as raw material from 89L of culture supernatant.

20

EXAMPLE 3

Toxicity, localization and clearance of CH₂-deleted chimeric BR96 was tested in vivo as follows.

25

Three dogs received 400 mg/m² of cBR96-A, the CH₂ deletion mutant of chimeric BR96, and two received chimeric BR96. Both molecules had been mildly reduced and alkylated. This is required to prevent dimerization of the deletion mutant into a tetravalent form. Both control dogs experienced the typical GI toxicity and none of

the three receiving the mutant displayed any toxicity. The control dogs and two of the test dogs were sacrificed at 1 hr to obtain duodenal tissue to measure antibody localization. Both control dogs had grossly visible GI pathology, and the test dogs had normal appearing GI tissue. The third dog has continued to show no signs of toxicity.

Results: A significant amount of localization of the CH₂ deleted cBR96 (cBR96-A) occurred to the GI tract in dogs treated with 400 mg/m², although the intact chiBR96 localized slightly better. The levels of localization indicate that roughly equivalent amounts of intact and CH₂ deleted cBR96 was delivered to the GI tract in these dogs.

Table 5. Localization of cBR96 to GI tissue.

Group	Animal	Specific	mean
		Localization	
	#271	155	
cBR96			135
	#272	114	
	#273	126	· ·
cBR96-A			89
	#274	52	

15

5

Using the mean level of specific localization, an amount of cBR96-A equivalent to at least 66% of the amount of cBR96 was delivered to the target organ of toxicity, the duodenum. Based on the dose ranging done with cBR96 in dogs (some clinical signs of toxicity seen at doses of 10 mg/m²), even if this difference is real, it could

not explain the difference between significant toxicity and no toxicity, evaluation to date indicated that dogs treated with cBR96-A had no toxicity, pending microscopic histopathologic examination. This evaluation was based on analysis of 2 frozen blocks per dog and 2 sections per block. Replicates were quite good. We also ran historical frozen tissues from dogs treated with native cBR96 or F(ab)2/BR96 and the levels of localization for those tissues were 110 and 0, respectively, consistent with our previous data.

Assuming that there is no toxicity at marginally higher (2X) doses of cBR96-A,

these data indicate that the CH₂ domain is associated with the induction of acute
gastroenteropathy, and that the removal of this domain prevents the induction of
gastroenteropathy mediated by BR96.

This study confirms the results showing that F(ab')2 is not toxic in the dog model
and that the toxicity is mediated by the constant region. The CH₂ deletion mutant is
a candidate for targeting agents clinically. Because of the very long half-life of
chimeric BR96, some decrease in the mutant's half-life should be acceptable.

Figure 1 shows the measurement of the clearance of the cBR96-A in high Le^Y

expressing dogs. The study used chimeric versus constant region mutant of cBR96
2.

CBR96-2 did clear faster than the chimeric BR96. The localization of cBR96-A to the gastrointestinal epithelium is not significantly affected by this more rapid clearance. More than enough of the cBR96-A localized to have caused toxicity.

Discussion: The constant region of chimeric IgG is responsible for the GI toxicity seen in clinical trials, e.g. with chiBR96-dox. The GI toxicity seen in the dog model is very similar to the clinical toxicity. Both in man and dog, administration of the

unconjugated antibody mediates an acute GI toxicity characterized by rapid onset of vomiting, often with blood.

In man the bleeding is limited to the fundus of the stomach, causing erosion of the superficial mucosa of the stomach. Although the pathology of the wound is limited and resolves, the extreme nature of the nausea and vomiting, unrelieved by antiemetics, defines it as the dose-limiting toxicity.

This toxicity is mediated in man and dog by the antibody molecule alone. At higher doses of the antibody-dox conjugate, additional toxicity is seen in the dog model, probably due to doxorubicin. Although the intact IgG of BR96 causes toxicity in dog and man, the F(ab')2 molecule (divalent and lacking only in the constant region) is not toxic in dogs. This finding has motivated our attempts at high levels, and improves the affinity and specificity of BR96 for tumor antigen.

The CH₂ domain is known to mediate complement and FcR binding. It was not known that structural alteration of the CH₂ domain would result in immunoglobulin-induced toxicity inhibition.

20 Toxicology study of hBR96-2B

15

The toxicology study of hBR96-2B in high Lewis Y expressor dogs (n=2) showed that a dose of 400 mg/m² did not cause hematemesis nor bloody stools, in contrast to BR96 which consistently causes one or both signs. A dog sacrificed at 24 hrs had normal gross appearance of the GI tract, again in marked contrast to chimeric BR96 which causes hemorrhagic lesions and mucosal erosions.

EXAMPLE 4

The polymerase chain reaction (PCR) is a widely used and versatile technique for the amplification and subsequent modification of immunoglobulin genes. The 5 rapidity and accuracy with which antibody genes can be modified in vitro has produced an assortment of novel antibody genes can be modified in vitro has produced an assortment of novel antibodies. For example, PCR methods have been used for engineering antibodies with increased affinity to antigen, for "humanizing" antibodies, and for modulating effector function (Marks, J.D., A.D. Griffiths, M. Malmqvist, T. Clackson, J.M. Bye and G. Winter. 1992. Bypassing immunization: high affinity human antibodies by chain shuffling. Bio/Technology 10:779-783; Rosok, M.J., D.E. Yelton, L.J. Harris, J. Bajorath, K.-E. Hellstrom, I. Hellstrom, G.A. Cruz, K. Kristensson, H. Lin, W.D. Huse and S.M. Glaser. 1996. A combinatorial library strategy for the rapid humanization of anticarcinoma BR96 Fab. J. Biol. Chem. 271:22611-22618; Morgan, A.N., D. Jones, A.M. Nesbitt, L. Chaplin, M.W. Bodmer and S. Emtage. 1995. The N-terminal end of the CH2 domain of chimeric human IgG1 anti-HLA-DR is necessary for Clg. FcyRI and FcyRIII binding. Immunology. 86:319-324).

As part of a more comprehensive study, we desired to introduce various site specific mutations in the CH₂ constant domain of human IgG₁. Six specific amino acid residues distributed throughout the CH2 domain previously identified to play a role in immune effector function were marked as targets for mutagenesis (Morgan, A.N., D. Jones, A.M. Nesbitt, L. Chaplin, M.W. Bodmer and S. Emtage. 1995. The N-terminal end of the CH2 domain of chimeric human IgG1 anti-HLA-DR is necessary for Clq, FcγRI and FcγRIII binding. Immunology. 86:319-324; Duncan, A.R. and G. Winter. 1988. The binding site for Clq on IgG. Nature 332:738-740; Tao, M.-H., R.I.F. Smith and S.L. Morrison. 1993. Structural features of human immunoglobulin G that determine isotype-specific differences in complement

activation. J.Exp.Med. 178:661-667). five of the six residues were grouped into two clusters-one cluster consisting of two residues, two amino acids apart (Location 1, or L1); and a second cluster consisting of three residues spanning a sequence of five amino acids (L2). The remaining amino acid position (L3) made for the total of six residues. We were interested in constructing a panel of mutant CH₂ domain IgGs consisting of each L mutation by itself as well as in combination with other L mutants (e.g., L1; L1; and L2; L1, L2 and L3; etc.).

Various in vitro methods have been described where PCR is used to simultaneously introduce distally located site-specific mutations within a gene sequence (Ho, S.N., 10 H.D. Hunt, R.M. Horton, J.K. Pullen and L.R. Pease. 1989. Site-directed mutagenesis by overlap extension. Gene 77:51-59; Ge, L. and P. Rudolpf. 1996. Simultaneous introduction of multiple mutations using overlap extention PCR. BioTechniques 22:28-30). Alternatively, an in vivo procedure termed recombination PCR (RPCR) has also successfully been used for rapidly and efficiently generating distally located site-specific mutations (Jones, D.H. and S.C. Winistorfer. 1993. Use of polymerase chain reaction for making recombinant constructs. p.241-250. In B.A. White (Ed.), Methods in Molecular Biology, Vol. 15. Humana Press Inc., Totowa, NJ, Jones, D.H. And B.H. Howard. 1991. A rapid method for recombination and site-specific mutagenesis by placing homologous ends on DNA 20 using polymerase chain reaction. BioTechniques 10:62-66). RPCR uses E. Coli's recombination machinery to generate intact circular recombinant plasmids from a transfected mixture of linear PCR-generated product and linearized vector. In vivo recombination is mediated through the joining of nucleotide sequences designed into the 5' ends of both PCR primers that are homologous to DNA sequences encoded by the vector. In this report we describe an extension of the RPCR procedure for simultaneously introducing complex combinations of mutations into an antibody CH₂ domain.

Humanized BR96 variable region heavy and light chain genes, previously cloned and co-expressed as an assembled active Fab fragment in an M13 phage expression vector, provided the starting material (Rosok, M.J., D.E. Yelton, L.J. Harris, J. Bajorath, K.-E. Hellstrom, I. Hellstrom, G.A. Cruz, K. Kristensson, H. Lin, W.D. Huse and S.M. Glaser. 1996. A combinatorial library strategy for the rapid humanization of anticarcinoma BR96 Fab. J. Biol. Chem. 271:22611-22618). The heavy and light chain V genes were amplified by PCR from a single-stranded M13 DNA template and subcloned by in vivo recombination (Jones, D.H. And B.H. Howard. 1991. A rapid method for recombination and site-specific mutagenesis by placing homologous ends on DNA using polymerase chain reaction. BioTechniques 10 10:62-66) into vectors pD17-hGla and pD16-hCk, to form pBR96-hGla and pBR96-hCk respectively. pD17-hG1a and pD16-hCk are eukaryotic immunoglobulin expression vectors derived from pcDNA3 (Invitrogen, San Diego, CA). The plasmid pBR96-hG1a was further modified by site-directed mutagenesis to introduce two Eco47-III restriction sites flanking the immunoglobulin hinge-CH₂-15 CH₂ domains using standard procedures. The recipient vector was then prepared by digesting pBR96-hG1a with Eco47-III, isolating the vector backbone by agarose gel electrophoresis followed by extracting the vector DNA from the excised gel slice

20

The strategy for introducing multiple mutations within the immunoglobulin CH₂ gene, shown in Figure 24, relies on the *in vivo* homologous recombination of several independently amplified PCR products with each other as well as with the pBR96-hG1a vector DNA. For introducing mutations at two distal locations two PCR products are synthesized (Figure 24B). One end of each PCR product is for recombining with an homologous end of the linear vector, and the other end, encoding the mutation(s) of interest, is for recombining with the neighboring PCR product. As shown in Figure 24B, additional distally-located mutations can be introduced into a target sequence by increasing the number of PCR products

using the Qiagen Gel Extraction kit (Qiagen, Chatsworth, CA).

proportionately. The recombination of neighboring PCR products always occurs across the regions containing the desired mutations, therefore the oligonucleotide primers encoding these ends (e.g., A1, A2) contain complementary mutant residues. The mutagenic PCR primers contain at least 15 nucleotides of wild-type sequence flanking each side of the mutant residues for either priming the polymerization reaction or mediating recombination. Two 49-nucleotide long PCR sense and antisense primers (Rs and Ra) contain sequences for recombining with the end regions of the Eco47-III digested pBR96-hG1a vector.

Each L mutation was amplified in a separate PCR reaction. The reaction conditions 10 were 250 ng intact pBR96-hG1a DNA template, 10 ul of 1X Pfu buffer (Stratagene, Inc. San Diego, CA), 10 nmol dNTPs, 200ng each of the appropriate PCR primers, 10% dimethysulfoxide (ATCC, Rockville, MD) and 2.5 units cloned Pfu DNA polymerase in a 100ul reaction volume. Samples were first denatured at 95° C for 5 min, cooled to 45°C for 5 min, and extended at 72°C for 1 min followed by 25 15 cycles of denaturation at 94°C for 45 sec, annealing at 45°C for 45 sec, extension at 72°C for 1 min/kb, followed by a final extension at 72°C for 7 min in a Perkin-Elmer DNA Thermal Cycler (Norwalk, CT). The amplified products were purified from a 1% agarose gel, extracted with Qiagen Gel Extraction kit and the recovered DNA quantitated. 50 ng of each PCR product was mixed with 25 ng of the Eco47-20 III digested pBR96-hG1a vector, transfected into Max competent E. coli DH5α according to the manufacturer's procedure (GIBCO BRL/Life Technologies, Gaithersburg, MD), and the entire transfection reaction plated onto selective LB agar plates containing 100 ug/ml ampicillin.

25

The results of several cloning experiments are summarized in the Table that follows. Typically the transformations produced from 80 to 200 bacterial colonies. Individual colonies were selected and grown overnight in 2 ml liquid cultures for isolation of miniprep plasmid DNA (Qiagen) and analysis by Eco47-III restriction

endonuclease mapping. Among 24 independent transformants analyzed from triple homologous recombination events (two PCR products plus vector) 11 clones contained the predicted 1.4 kpb DNA insert.

Figure 25 shows a sample diagnostic restriction analysis of DNA prepared from clones derived from quadruple homologous recombination events (three PCR products plus vector). Additional sampling of clones resulting from quadruple recombination yielded a cloning efficiency of 29% (7 clones containing inserts/24 clones sampled). At this point, due to the small sampling sizes, we do not know whether the differences in the cloning efficiencies observed between the triple and quadruple recombination events are meaningful.

To evaluate the expression of Le7 -binding activity of the CH2 mutant IgGs, miniprep DNAs from 6 clones derived from the triple recombination reaction and 6 clones derived from the quadruple recombination reaction exhibiting the predicted 15 diagnostic Eco47-III restriction patterns were isolated, mixed with pBR96- hCk DNA and used to co-transfect COS7 cells. 48 hour spent supernatants from 3 ml cultures were assayed for total IgG production and for Le7 binding activity by enzyme-linked immunosorbent assay (EIA) as described (Yelton, D.E., M.J. Rosok, G.A. Cruz, W.L. Cosand, J. Bajorath, I. Hellstom, K.-E. Hellstorm, W.D. Huse and 20 S.M. Glaser. 1995. Affinity maturation of the BR96 anti-carcinoma antibody by codon-based mutagenesis. J.Immunol. 155:1994-2004). All twelve cultures were found to secrete approximately 2-3 ug/ml Le⁷ -reactive IgG. The spectrum of Le⁷ binding activities were all similar to that of native humanized BR96 IgG indicating that the homologously recombined antibodies did not acquire any gross mutations that could affect antigen binding. To confirm that the desired CH2 mutations had been incorporated, and to evaluate the recombined genes for misincorporated nucleotides, four of the clones producing functional antibody were sequenced using Sequenase Version 2 DNA Sequencing Kit (United States Biochemical). One clone

was found to contain a single nucleotide change within the forward PCR primer used for mediating recombination with vector DNA. We are uncertain whether this error occurred during chemical synthesis of the oligonucleotide primer or is a result of misincorporation during the PCR reaction, despite the fact that we used a thermostable polymerase with proofreading activity.

A RPCR procedure for homologously recombining up to three separate PCR-generated mutated antibody sequence products into a eukaryotic expression vector for the rapid construction of engineered IgG molecules is described herein. The advantage of this approach is the ability to simultaneously introduce multiple distally-located mutations with PCR products synthesized by a single round of PCR. Recombinant DNAs are produced with a reasonably high cloning efficiency and fidelity of correct nucleotide sequences. The ability to efficiently rejoin several distinct PCR products should permit combinatorial strategies for constructing complexly mutated protein domains as well as broadening the number and location of desired mutations.

Analysis of transformants generated by multiple-fragment RPCR.

Mutant IgGs	PCR	HR ^a events	Colonies	Cloning
Constructed	Fragments in reaction		Analyzed	Efficiency ^b
2	2	triple	24	45%
2	3	quadruple	24	33%

^aHR-homologous recombination

5

^bCloning efficiency (number of clones containing 1.4kbp insert/total number of colonies

EXAMPLE 5

This example provides two methods for introducing site specific mutations into the 5 CH2 domain of human IgG1 constant region containing vectors.

One method involves PCR amplification of a segment or segments of the constant region, wherein mutations are introduced using appropriately constructed oligonucleotides. The vector receiving the fragment(s) is digested with a restriction enzyme to linearize the vector. PCR amplification primers are designed so that the 5' ends of the PCR fragments can hybridize to the DNA sequence of the vectors. If more than one PCR fragment is amplified, then common sequences to the two fragments are introduced by oligonucleotides. Bacteria are transfected with the PCR fragments and with the digested vector. The fragments and vector can recombine by homologous recombination using the bacteria's recombination machinery. Bacterial colonies are selected and the DNA is analyzed by size and restriction map as a preliminary determination that the vector and fragment(s) recombined correctly. Correct insertion of fragments with the mutations is confirmed by dideoxynucleotide sequence analysis. DNA is then introduced into mammalian cells as described for the CH2 deleted antibody, and the expressed antibody analyzed for binding and functional activity.

By way of example, mutations Leu to Ala at residue 235 in CH2 and Gly to Ala at residue 237 were introduced by the procedure disclosed in Example 4. The heavy chain vector used for this procedure was pD17-hG1a, similar to pD17-BR96 vector described herein except that humanized V regions (Rosok, M.J., D.E. Yelton, L.J. Harris, J. Bajorath, K-E. Hellstrom, I, Hellstrom, G.A. Cruz, K. Kristensson, H. Lin, W.D. Huse, and S.M. Glaser, 1996. J. Biol. Chem 271 37:22611-22618) with three affinity mutations (H1, H2, and H3 mutations) were substituted.

pBR96-hG1a contains two Eco47-III restriction sites flanking the Ig hinge-CH2-CH3 domains. The recipient vector was prepared by (1) digesting pBR96-hG1a with Eco47-III, (2) isolating the vector by agarose gel electrophoresis, and (3) extracting the vector DNA from the excised gel slice using the Qiagen Gel Extraction kit (Qiagen, Chatsworth, CA). To introduce mutations at a single location, such as for positions 235 and 237, two PCR products were synthesized.

To introduce two distally located mutations, such as for mutant F (also referred to herein as hBR96-2F) with mutations at 235, 237, 331, requires 3 PCR products. The recombination of neighboring PCR products occurs across the regions containing the desired mutations, therefore the oligonucleotide primers encoding these ends contain complementary mutant residues. The mutagenic PCR primers contain at least 15 nucleotides of wild-type sequence flanking each side of the mutant residues for either priming the polymerization reaction or mediating recombination. Two 49-nucleotide long PCR sense and anti-sense primers containing sequences for recombining with the end regions of the *Eco*47-III digested pBR96-hG1a vector.

PCR amplification used 250 ng intact pBR96-hG1a DNA template, 10 μl of 10X Pfu

buffer (Stratagene, Inc., San Diego, CA), 10 nmol dNTPs, 200 ng each of the
appropriate PCR primers, 10% dimethylsulfoxide (ATCC, Rockville, MD) and 2.5

units cloned Pfu DNA polymerase (Stratagen, Inc. San Diego, CA) in 100 μl
reaction. Samples were denatured at 95°C for 5 min, annealed at 45°C for 5 min,
and extended at 72°C for 1 min followed by 25 cycles of denaturation at 94°C for 45

sec, annealing at 45°C for 45 sec, extension at 72°C for 1 min/kb, and a final
extension at 72°C for 7 min. The amplified products were purified from a 1%
agarose gel, extracted with the Qiagen Gel Extraction kit and quantitated. 50 mg of
each PCR product was mixed with 25 ng of the Eco47-III digested pBR96-hG1a
vector and transfected in E.coli MAX Efficiency DH5α™ according to the

manufacturer's instructions (GIBCO BRL/Life Technologies, Gaithersburg, MD). The entire transfection reaction was plated onto LB agar plated containing 100 μg/ml ampicillin.

- Bacterial colonies were selected and grown overnight at 37° C in 2 ml liquid cultures. DNA was isolated and analyzed by Eco47-III restriction endonuclease mapping. Clones with the correct size insert were sequenced (Sequenase Version 2, U.S. Biochemical Corp., Cleveland, OH).
- The second method for introducing site specific mutations into the CH₂ domain of human IgG1 involved the method of Kunkel (1987 Methods Enzymology, supra). For this procedure pD17-hG1b DNA with the F1 origin of replication was introduced into electrocompetent E. coli CJ236 dut-ung- (Bio-Rad Laboratories, Hercules, CA) by electroporation according to manufacturer's instructions. PD17-
- hG1b is a vector having a constant region but no variable region. The F1 ori site allows treatment of this vector as a phagemid.
- Bacteria containing the plasmid were selected by ampicillin resistance. Single stranded uridinylated DNA was prepared using the Muta-Gene Phagemid In Vitro

 Mutagenesis Version 2 protocol (Bio-Rad). Mutations were introduced by site-directed mutagenesis with the appropriate antisense oligonucleotide. For molecules with mutations at more than one location, mutations were introduced by either of the two methods discussed above. One method would be to (1) prepare one mutant, for example, mutant 2C (also referred to herein as BR96-2C) with the mutations at residues 318, 320, 322, (2) isolate ssDNA, and (3) introduce a second mutation set with the appropriate anti-sense oligonucleotide. The second method would be to anneal two antisense oligonucleotides with the same uridinylated ssDNA and screen for mutants with both sets of changes. Mutant 2H (hBR96-2H) was also prepared

by a combination of thse methods.

The V region of humanized BR96-2 heavy chain was introduced by the homologous recombination method described above in pD17-hJm14.H1. The pD17-hJm14.H1 plasmid contains the BR96 humanized variable region with the H1/H2/H3

mutations and the plasmid was used to transfect mutant sequences into mammalian cells. The pD17G1b vector containing the Fc mutation(s) was digested with Nhel for 3 hr at 37° C and the DNA isolated by methods described above. Insertion of the V region into the vector was determined by size and restriction enzyme mapping and confirmed by sequence analysis.

10

Transient expression of whole antibodies was performed by transfection of COS cells. For production of antibody, stable transfections of CHO cells were performed (see description of deleted CH2 mutant). All mutants were purified from CHO culture supernatants by protein A chromatography.

15

The oligonucleotide primers homologous to the vector and used to introduce the constant regions mutations were as follows:

Oligonucleotides homologous to vector sequences:

Sens(sense)CH2 E47-3-5: CAG GGA GGG AGG GTG TCT GCT GGA AGC

20 CAG GCT CAG CGC TGA CCT CAGA

D CH2 E47-3 A (antisense): GGA AAG AAC CAT CAC AGT CTC GCA GGG GCC CAG GGC AGC GCT GGG TGC TT

Oligonucleotides to mutate Leu235 to Ala and Gly237 to Ala (underlined sequences show sites of mutation):

Antisense CH2 L235-G237/aa: GAA GAG GAA GAC TGA CGG TGC CCC CGC GAG TTC AGG TGC TGA GG

SensCH2 L235-G237/AA: CCT CAG CAC CTG AAC TCG CGG GGG CAC CGT CAG TCT TCC TCT TC

Oligonucleotides to mutate Glu318, Lys320, Lys322 to Ser

Antis(antisense)CH2 EKK/SSS-2: CTG GGA GGG CTT TGT TGG AGA CCG

AGC ACG ACT ACG ACT TGC CAT TCA GCC

5 Oligonucleotides to mutate Pro331 to Ala:

Antis CH2 P331/A/3: GAT GGT TTT CTC GAT GGC GGC TGG GAG GGC Sense CH2 P33/A: GCC CTC CCA GCC GCC ATC GAG AAA ACC ATC Alternative antisense oligo to introduce Ala at 331 by site-directed mutation: CH2P331A: GAT GGT TTT CTC GAT AGC GGC TGG GAG GGC TTT G

10

15

Oligonucleotides to mutate Glu318 to Ser, Lys320 to Ser, Lys322 to Ser, and Pro331 to Ala:

Antis CH2 EKKP/SSA-6: GAT GGT TTT CTC GAT GGC GGC TGG GAG GGC TTT GTT GGA GAC CGA GCA CGA GTA CGA CTT GCC ATT CAG CCA GTC CTG GTG

Sense CH2 EKKP/SSA-6: CAC CAG GAC TGG CTG AAT GGC AAG TCG TAC TCG TGC TCG GTC TCC AAC AAA GCC CTC CCA GCC GCC ATC GAG AAA ACC ATC

20

In vitro Assays of the Mutants

Results of the CDC demonstrate that mutant hBR96-2B has approximately 10 fold less activity than the control hBR96-1 (two affinity mutations, one in H2 and one in H3, refer to previous patent (Figure 20)). The mutants that have the least ability to kill cells in the presence of complement is hBR96-2C with the triple mutations at positions 318, 320, and 322 and the hBR96-2H mutant (least cytotoxic antibodies in the panel) which contains all six mutations at the three different locations. ADCC activity was most affected by the CH2 deleted hBR96-2 molecule (Figure 21).

hBR96-2B and -2H lost between 100 and 1000 fold activity to kill in the presence of effector cells. In the ADCC assay the hBR96-2B molecule also lost approximately 10 fold activity (Figure 21).

5 Figures 26-28 provide the amino acid sequences for the heavy chain variable region for both chimeric and humanized BR96 having the H1, H2, and H3 mutations. The amino acid sequence for the light chain variable region is known and methods for generating it are found in PCT Application No. 95/305444. Additionally provided is the amino acid sequence for the IgG1 constant region. Mutations in the constant region are marked.

49

SEQUENCE LISTING

_	(1) GENERAL INFORMATION
5	(i) APPLICANT: Bristol-Myers Squibb Co.
10	(ii) TITLE OF THE INVENTION: A METHOD FOR INHIBITING IMMUNOGLOBULIN-INDUCED TOXICITY FROM THE USE OF IMMUNOGLOBULINS IN THERAPY AND IN VIVO DIAGNOSIS
	(iii) NUMBER OF SEQUENCES: 13
15	(A) ADDRESSEE: Merchant & Gould(B) STREET: 11150 Santa Monica Blvd., Suite 400(C) CITY: Los Angeles
20	(D) STATE: CA (E) COUNTRY: USA (F) ZIP: 90025
25	(v) COMPUTER READABLE FORM: (A) MEDIUM TYPE: Diskette (B) COMPUTER: IBM Compatible (C) OPERATING SYSTEM: DOS (D) SOFTWARE: FastSEQ Version 2.0
30	(vi) CURRENT APPLICATION DATA:(A) APPLICATION NUMBER: PCT/US97/(B) FILING DATE: 01-AUG-1997(C) CLASSIFICATION:
35	(vii) PRIOR APPLICATION DATA: (A) APPLICATION NUMBER: 60/023,033 (B) FILING DATE: 02-AUG-1996
40	<pre>(viii) ATTORNEY/AGENT INFORMATION: (A) NAME: Adriano, Sarah B (B) REGISTRATION NUMBER: 34,470 (C) REFERENCE/DOCKET NUMBER: 30436.43WOU1</pre>
45	(ix) TELECOMMUNICATION INFORMATION: (A) TELEPHONE: 310-445-1140 (B) TELEFAX: 310-445-9031 (C) TELEX:
50	(2) INFORMATION FOR SEQ ID NO:1:
55	 (i) SEQUENCE CHARACTERISTICS: (A) LENGTH: 36 base pairs (B) TYPE: nucleic acid (C) STRANDEDNESS: single (D) TOPOLOGY: linear

	(ii) MOLECULE TYPE: cDNA	
	(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:	
5	TGGCACCGAA AGCTTTCTGG GGCAGGCCAG GCCTGA	36
	(2) INFORMATION FOR SEQ ID NO:2:	
0	 (i) SEQUENCE CHARACTERISTICS: (A) LENGTH: 57 base pairs (B) TYPE: nucleic acid (C) STRANDEDNESS: single (D) TOPOLOGY: linear 	
.5	(ii) MOLECULE TYPE: cDNA	
	(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:	
20	TCCGGACATG TTGGTACCCA CGTGGTGGTC GACGCTGAGC CTGGCTTCGA GCAGACA	57
20	(2) INFORMATION FOR SEQ ID NO:3:	
25	 (i) SEQUENCE CHARACTERISTICS: (A) LENGTH: 55 base pairs (B) TYPE: nucleic acid (C) STRANDEDNESS: single (D) TOPOLOGY: linear 	
30	(ii) MOLECULE TYPE: cDNA (xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:	
	GTCGACCACC ACGTGGGTAC CAACATGTCC GGAGCCACAT GGACAGAGGC CGGCT	55
35	(2) INFORMATION FOR SEQ ID NO:4:	
	(i) SEQUENCE CHARACTERISTICS: (A) LENGTH: 30 base pairs (B) TYPE: nucleic acid	
40	(C) STRANDEDNESS: single (D) TOPOLOGY: linear	
	(ii) MOLECULE TYPE: cDNA	
45	(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:	30
	CTGGTTCTTG TTCATCTCCT CTCTAGATGG (2) INFORMATION FOR SEQ ID NO:5:	
50	(i) SEQUENCE CHARACTERISTICS:	
	(A) LENGTH: 36 base pairs (B) TYPE: nucleic acid (C) STRANDEDNESS: single	
55	(D) TOPOLOGY: linear	
	(ii) MOLECULE TYPE: cDNA	
	(vi) SEQUENCE DESCRIPTION: SEO ID NO:5:	

	ACCATGGTCG ACCTCAGACC TGCCAAGAGC CATATC	36
_	(2) INFORMATION FOR SEQ ID NO:6:	
5 ·	(i) SEQUENCE CHARACTERISTICS:	
	(A) LENGTH: 39 base pairs	
	(B) TYPE: nucleic acid	
10	(C) STRANDEDNESS: single	
10	(D) TOPOLOGY: linear	
	(ii) MOLECULE TYPE: cDNA	
	(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:	
15	CATGGTCACG TGGTGTGCC CTGGATGCAG GCTACTCTA	39
	(A) ANDORMATION FOR SEC ID NO. 7.	
	(2) INFORMATION FOR SEQ ID NO:7:	
20	(i) SEQUENCE CHARACTERISTICS:	
	(A) LENGTH: 49 base pairs (B) TYPE: nucleic acid	
	(C) STRANDEDNESS: single	
	(D) TOPOLOGY: linear	
25	•	
	(ii) MOLECULE TYPE: cDNA	
	(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:	
30	CAGGGAGGGA GGGTGTCTGC TGGAAGCCAG GCTCAGCGCT GACCTCAGA	49
	(2) INFORMATION FOR SEQ ID NO:8:	
	(i) SEQUENCE CHARACTERISTICS:	
35	(A) LENGTH: 50 base pairs	
	(B) TYPE: nucleic acid	
	(C) STRANDEDNESS: single	
	(D) TOPOLOGY: linear	
40	(ii) MOLECULE TYPE: cDNA	
	(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:	
	GGAAAGAACC ATCACAGTCT CGCAGGGGCC CAGGGCAGCG CTGGGTGCTT	50
45	(2) INFORMATION FOR SEQ ID NO:9:	
	(2) Intomation tox day is not?	
	(i) SEQUENCE CHARACTERISTICS:	
50	(A) LENGTH: 8691 base pairs (B) TYPE: nucleic acid	
50	(C) STRANDEDNESS: single	
	(D) TOPOLOGY: linear	
	(ii) MOLECULE TYPE: cDNA	
55	(II) MODECORE LIFE: CDMA	
	(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:	
	GACGGATCGG GAGATCTGCT AGGTGACCTG AGGCGCGCCG GCTTCGAATA GCCAGAGTAA	60
	COTTTTTTT TAATTITATT TTATTTTATT TTTGAGATGG AGTTTGGCGC CGATCTCCCG	120

						*************	180
	ATCCCCTATG	GTCGACTCTC	AGTACAATCT	GCTCTGATGC	CGCATAGTTA	MGCCMGIMIC	240
		ウサンサンサンフサンス	CACCTCGCTG	AGTAGTGCGC	GAGCAAAATT	IWWQCIWCWW	300
		CTTCACCGAC	AATTGCATGA	AGAATCTGCT	TAGGGTTAGG	CGTTTTGCGC	
		THE TRACE CONTRACT OF THE PROPERTY OF THE PROP	AGATATACGC	GTTGACATTG .	ATTATIGACI	AGITATTAAT	360
5		MA COCCOTON	ጥተከርንሞተጋል ሞል	GCCCATATAT	GGAGTTCCGC	GITACATAAC	420
-		TOTOCOCOCO	GGCTGACCGC	CCAACGACCC	CCGCCCATTG	ACGICAATAA	480
		TCCCATAGTA	ACCCCAATAG	GGACTTTCCA	TTGACGTCAA	TGGGTGGACT	540
		N N CTCCCCC N C	TTCCCACTAC	ATCAAGTGTA	TCATATGCCA	AGTACGCCCC	600
		CNATCACCCT	AAATGGCCCG	CCTGGCATTA	TGCCCAGTAC	ATGACCITAT	660
10		TRACTORICACE AC	TACATCTACG	TATTAGTCAT	CGCTATTACC	AIGGIGAIGC	720
10		TAADTADAT	CCCCCTCCAT	AGCGGTTTGA	CTCACGGGGA	TTTCCAAGTC	780
		TC A CCTC A A T	CCCACTTTGT	TTTGGCACCA	AAATCAACGG	GACTTTCCAA	840
	mamaama a	CNACTCCCCC	CCATTGACGC	AAATGGGCGG	TAGGCGTGTA	CGGTGGGAGG	900
		CACACCTCTC	TCCCTAACTA	GAGAACCCAC	TGCTTACTGG	CITATCGAAA	960
	TCTATATAAG	TCACTATAGG	CACACCCAAG	CTTGGTACCA	ATTTAAATTG	ATATCTCCTT	1020
15	TTAATACGAC	TCTCTAGATA	ACCCCTCAAT	CGATTGGAAT	TCTTGCGGCC	GCTTGCTAGC	1080
	AGGTCTCGAG	TTGTGGTTAA	CCTTGGTCCT	TCCTTGTCCT	TGTTTTAAAA	GGTGTCCAGT	1140
	CACCATGGAG	TCTGGTGGAG	GC11GG1CC1	CCTTAGTGCA	GCCTGGAGGG	TCCCTGAAAG	1200
	GTGAAGTGAA	AACCTCTGGA	TC1GGGGGGG	CTCACTATTA	CATGTATTGG	GTTCGCCAGA	1260
	TCTCCTGTGT	GAGGCTGGAG	TICACITICA	ACATTACTCA	AGGTGGTGAT	ATAACCGACT	1320
20	CTCCAGAGAA	GAGGCTGGAG	TGGGTCGCAT	MCMITAGICA	CANTOCCANG	AACACCCTGT	1380
	ATCCAGACAC	TGTAAAGGGT	CGATTCACCA	TCTCCAGAGA	CANTOCCANO	GCAAGAGGCC	1440
	ACCTGCAAAT	GAGCCGTCTG	AAGTCTGAGG	ACACAGCCAI	MODOCOTO CO	CTCTCTCTAG	1500
	TGGACGACGG	GGCCTGGTTT	GCTTACTGGG	GCCAAGGGAC	TCIGGICACG	ACCTCTGGGG	1560
	CTAGCACCAA	GGGCCCATCG	GTCTTCCCCC	TGGCACCCTC	CTCCAAGAGC	ACCICIOGGG	1620
25	GCACAGCGGC	CCTGGGCTGC	CTGGTCAAGG	ACTACTTCCC	CGAACCGGTG	CACTCCTCAC	1680
	GGAACTCAGG	CGCCCTGACC	AGCGGCGTGC	ACACCTTCCC	GGCTGTCCTA	AGICCICAG	1740
	GACTCTACTC	CCTCAGCAGC	GTGGTCACCG	TGCCCTCCAG	CAGCTTGGGC	ACCCAGACCI	1800
	ACATCTGCAA	CGTGAATCAC	AAGCCCAGCA	ACACCAAGGT	GGACAAGAAA	GTTGGTGAGA	1860
	GGCCAGCACA	GGGAGGGAGG	GTGTCTGCTG	GAAGCCAGGC	TCAGCGCTCC	TGCCTGGACG	1920
30	CATCCCGGCT	ATGCAGCCCC	AGTCCAGGGC	AGCAAGGCAG	GCCCGTCTG	CCTCTTCACC	1920 1980
	CGGAGGCCTC	TGCCCGCCCC	ACTCATGCTC	ACGGAGAGGG	TCTTCTGGCT	TTTTCCCCAG	
	a concentration of	CCCXCXCCCT	AGGTGCCCCT	AACCCAGGCC	CTGCACACAA	AGGGGCAGGT	2040
	COMOGCOTC)	CACCTGCCAA	CACCCATATO	CGGGAGGACC	CTGCCCCTGA	CCTAAGCCCCA	2100
	GGGGD 3 3 CCC	CNARCTCTCC	ACTCCCTCAG	CTCGGACACC	TTCTCTCCTC	CCAGATTCCA	2160
35	CONTRACTOR	V-delated California	TGCAGAGCCC	AAATCTTGTG	ACAAAACTCA	CACATGCCCA	2220
	COCECCCAC	CTARCCCAGC	CCAGGCCTCG	CCCTCCAGCT	CAAGGCGGGA	CAGGIGCCCI	2280
	3 C3 CT3 CCCT	CCATCCACGG	ACAGGCCCCA	GCCGGGTGCT	GACACGTCCA	CCTCCATCTC	2340
	THE CT CACCA	CCTGAACTCC	TGGGGGGACC	: GTCAGTCTTC	CTCTTCCCCC	CAAAACCCCAA	2400
	CON CO COCOTO	አጥርአጥርጥርር	GGACCCCTGA	. GGTCACATGC	GTGGTGGTGG	ACGIGAGCCA	2460
40	CONNERCOCC	CACCTCAAGT	TCAACTGGTA	\ CGTGGACGGC	GTGGAGGTGC	ATAATGCCAA	2520
40	CACAAACCCC	CCCCACCACCACC	AGTACAACAC	CACGTACCGT	GTGGTCAGCG	TCCTCACCGI	2580
	GGDGGA GGA G	CACTCCCTCA	ATGGCAAGG	GTACAAGTGC	AAGGTCTCC	ACAAAGCCCT	2640
	GGGACCCCCC	ATCCACAAA	CCATCTCCAZ	A AGCCAAAGGT	GGGACCCGTC	GGGTGCGAGG	2700
	COCACATOCA	CACAGGCCGG	CTCGGCCCAC	CCTCTGCCCT	GAGAGTGACC	GCIGIACCAA	2760
45		MACACCCCAC	CCCCCAGAA	' CACAGGTGTA	CACCCTGCCC	CCATCCCGGG	2820
47	A TO A COTO A C	CANCANCOR	GTCAGCCTG	A CCTGCCTGGT	CAAAGGCTT	TATCCCAGCG	2880
	A CARCCOCCI	CANCINCER	AGCAATGGG	AGCCGGAGAA	CAACTACAAC	ACCACGCCTC	2940
	ACATOGCOG	CTCCCACCC	TCCTTCTTC	TCTACAGCAA	GCTCACCGT	GACAAGAGCA	3000
	CCGIGCIGGA	COCCARCOOC	TTCTCATGC	CCGTGATGCA	TGAGGCTCT	CACAACCACT	3060
50	GGTGGCAGCA	CACCCTCTCC	CTGTCTCCG	GTAAATGAGT	GCGACGGCC	GCAAGCCCCC	3120
50	ACACGCAGAA	A CAGCCICICC	CCCACCACC	A TOUTTOGCAC	GTACCCCCT	TACATACTTC	3180
	GCTCCCCGGC	, CICICOCOCI	TABACCACCA	C ACCCCTCCCC	TGGGCCCCT	GCGAGACTGTG	3240
	CCGGGCGCCC	AGUATGGAAA	- COCCOS CEC	T GAGGGGGGGG	TGGCATGAG	GAGGCAGAGC	3300
	ATGGTTCTT	CCACGGGTCA	COCCGAGTC	T GWOGCCIGHG	TGCCATGAG	CCCTAGGGTG	3360
	GGGTCCCAC	r GTCCCCACAC	TGGCCCAGG	C IGIGCAGGIC	, receience	CCTCCCTCC	3420
55	GGGCTCAGC	AGGGGCTGCC	CTCGGCAGG	G RECOCCERC	ACCOCCAGO	G CCCTCCCTCC	3480
	AGCAGCACC	r GCCCTGGGC1	GGGCCACGG	AAGCCCTAGG	- Unchronista	G GACAGACACA C TCCCGACCTC	3540
	CAGCCCCTG	CTCTGTAGGA	GACTGTCCT	G TTCTGTGAGG	. ACCCCTATO	C TCCCGACCTC	3600
	CATGCCCAC	r cgggggcate	CCTAGTCCA	T GTGCGTAGGC	MCMOGCCCT	C CCTCACCCAT	3660
	CTACCCCCA	C GGCACTAACO	CCTGGCTGC	C CTGCCCAGC	TUGUALUUG	C ATGGGGACAC	3000

```
AACCGACTCC GGGGACATGC ACTCTCGGGC CCTGTGGAGG GACTGGTGCA GATGCCCACA
                                                                          3720
     CACACACTCA GCCCAGACCC GTTCAACAAA CCCCGCACTG AGGTTGGCCG GCCACACGGC
                                                                          3780
     CACCACACAC ACACGTGCAC GCCTCACACA CGGAGCCTCA CCCGGGCGAA CTGCACAGCA
     CCCAGACCAG AGCAAGGTCC TCGCACACGT GAACACTCCT CGGACACAGG CCCCCACGAG
                                                                          3900
     CCCCACGCGG CACCTCAAGG CCCACGAGCC TCTCGGCAGC TTCTCCACAT GCTGACCTGC
 5
                                                                          3960
     TCAGACAAAC CCAGCCCTCC TCTCACAAGG GTGCCCCTGC AGCCGCCACA CACACACAGG
     GGATCACACA CCACGTCACG TCCCTGGCCC TGGCCCACTT CCCAGTGCCG CCCTTCCCTG
                                                                          4080
     CAGGACGGAT CAGCCTCGAC TGTGCCTTCT AGTTGCCAGC CATCTGTTGT TTGCCCCTCC
                                                                          4140
     CCCGTGCCTT CCTTGACCCT GGAAGGTGCC ACTCCCACTG TCCTTTCCTA ATAAAATGAG
                                                                          4200
     GAAATTGCAT CGCATTGTCT GAGTAGGTGT CATTCTATTC TGGGGGGTGG GGTGGGGCAG
10
                                                                          4260
     GACAGCAAGG GGGAGGATTG GGAAGACAAT AGCAGGCATG CTGGGGATGC GGTGGGCTCT
                                                                          4320
     ATGGCTTCTG AGGCGGAAAG AACCAGCTGG GGCTCTAGGG GGTATCCCCA CGCGCCCTGT
     AGCGGCGCAT TAAGCGCGGC GGGTGTGGTG GTTACGCGCA GCGTGACCGC TACACTTGCC
                                                                          4440
     AGCGCCCTAG CGCCCGCTCC TTTCGCTTTC TTCCCTTCCT TTCTCGCCAC GTTCGCCGGG
                                                                          4500
15
     CCTCTCAAAA AAGGGAAAAA AAGCATGCAT CTCAATTAGT CAGCAACCAT AGTCCCGCCC
                                                                          4560
     CTAACTCCGC CCATCCCGCC CCTAACTCCG CCCAGTTCCG CCCATTCTCC GCCCCATGGC
                                                                          4620
     TGACTAATTT TTTTTATTTA TGCAGAGGCC GAGGCCGCCT CGGCCTCTGA GCTATTCCAG
                                                                          4680
     AAGTAGTGAG GAGGCTTTTT TGGAGGCCTA GGCTTTTGCA AAAAGCTTGG ACAGCTCAGG
     GCTGCGATTT CGCGCCAAAC TTGACGGCAA TCCTAGCGTG AAGGCTGGTA GGATTTTATC
                                                                          4800
20
     CCCGCTGCCA TCATGGTTCG ACCATTGAAC TGCATCGTCG CCGTGTCCCA AAATATGGGG
                                                                          4860
     ATTGGCAAGA ACGGAGACCT ACCCTGGCCT CCGCTCAGGA ACGAGTTCAA GTACTŢCCAA
                                                                          4920
     AGAATGACCA CAACCTCTTC AGTGGAAGGT AAACAGAATC TGGTGATTAT GGGTAGGAAA
                                                                          4980
     ACCTGGTTCT CCATTCCTGA GAAGAATCGA CCTTTAAAGG ACAGAATTAA TATAGTTCTC
     AGTAGAGAAC TCAAAGAACC ACCACGAGGA GCTCATTTTC TTGCCAAAAG TTTGGATGAT
                                                                          5100
25
     GCCTTAAGAC TTATTGAACA ACCGGAATTG GCAAGTAAAG TAGACATGGT TTGGATAGTC
                                                                          5160
     GGAGGCAGTT CTGTTTACCA GGAAGCCATG AATCAACCAG GCCACCTTAG ACTCTTTGTG
      ACAAGGATCA TGCAGGAATT TGAAAGTGAC ACGTTTTTCC CAGAAATTGA TTTGGGGAAA
                                                                          5280
      TATAAACTTC TCCCAGAATA CCCAGGCGTC CTCTCTGAGG TCCAGGAGGA AAAAGGCATC
                                                                          5340
      AAGTATAAGT TTGAAGTCTA CGAGAAGAAA GACTAACAGG AAGATGCTTT CAAGTTCTCT
                                                                          5400
     GCTCCCCTCC TARAGCTATG CATTTTATA AGACCATGGG ACTTTTGCTG GCTTTAGATC
30
                                                                          5460
      TCTTTGTGAA GGAACCTTAC TTCTGTGGTG TGACATAATT GGACAAACTA CCTACAGAGA
      TTTAAAGCTC TAAGGTAAAT ATAAAATTTT TAAGTGTATA ATGTGTTAAA CTACTGATTC
      TAATTGTTTG TGTATTTTAG ATTCCAACCT ATGGAACTGA TGAATGGGAG CAGTGGTGGA
      ATGCCTTTAA TGAGGAAAAC CTGTTTTGCT CAGAAGAAAT GCCATCTAGT GATGATGAGG
      CTACTGCTGA CTCTCAACAT TCTACTCCTC CAAAAAAGAA GAGAAAGGTA GAAGACCCCA
35
                                                                          5760
      AGGACTITCC TICAGAATIG CTAAGITITI IGAGICATGC IGIGITIAGI AATAGAACTC
      TTGCTTGCTT TGCTATTTAC ACCACAAAGG AAAAAGCTGC ACTGCTATAC AAGAAAATTA
                                                                          5880
      TGGAAAAATA TTCTGTAACC TTTATAAGTA GGCATAACAG TTATAATCAT AACATACTGT
      TTTTTCTTAC TCCACACAGG CATAGAGTGT CTGCTATTAA TAACTATGCT CAAAAATTGT
                                                                          6060
40
      GTACCTTTAG CTTTTTAATT TGTAAAGGGG TTAATAAGGA ATATTTGATG TATAGTGCCT
      TGACTAGAGA TCATAATCAG CCATACCACA TTTGTAGAGG TTTTACTTGC TTTAAAAAAAC
                                                                          6120
      CTCCCACACC TCCCCCTGAA CCTGAAACAT AAAATGAATG CAATTGTTGT TGTTAACTTG
                                                                          6180
      TTTATTGCAG CTTATAATGG TTACAAATAA AGCAATAGCA TCACAAATTT CACAAATAAA
      GCATTTTTTT CACTGCATTC TAGTTGTGGT TTGTCCAAAC TCATCAATGT ATCTTATCAT
      GTCTGGATCG GCTGGATGAT CCTCCAGCGC GGGGATCTCA TGCTGGAGTT CTTCGCCCAC
      CCCAACTTGT TTATTGCAGC TTATAATGGT TACAAATAAA GCAATAGCAT CACAAATTTC
                                                                          6420
      ACAAATAAAG CATTTTTTC ACTGCATTCT AGTTGTGGTT TGTCCAAACT CATCAATGTA
      TCTTATCATG TCTGTATACC GTCGACCTCT AGCTAGAGCT TGGCGTAATC ATGGTCATAG
                                                                          6540
      CTGTTTCCTG TGTGAAATTG TTATCCGCTC ACAATTCCAC ACAACATACG AGCCGGAAGC
                                                                          6600
50
      ATAAAGTGTA AAGCCTGGGG TGCCTAATGA GTGAGCTAAC TCACATTAAT TGCGTTGCGC
                                                                          6660
      TCACTGCCCG CTTTCCAGTC GGGAAACCTG TCGTGCCAGC TGCATTAATG AATCGGCCAA
                                                                          6720
      CGCGCGGGGA GAGGCGGTTT GCGTATTGGG CGCTCTTCCG CTTCCTCGCT CACTGACTCG
                                                                           6780
      CTGCGCTCGG TCGTTCGGCT GCGGCGAGCG GTATCAGCTC ACTCAAAGGC GGTAATACGG
                                                                           6840
      TTATCCACAG AATCAGGGGA TAACGCAGGA AAGAACATGT GAGCAAAAGG CCAGCAAAAG
                                                                          6900
      GCCAGGAACC GTAAAAAGGC CGCGTTGCTG GCGTTTTTCC ATAGGCTCCG CCCCCCTGAC
55
      GAGCATCACA AAAATCGACG CTCAAGTCAG AGGTGGCGAA ACCCGACAGG ACTATAAAGA
      TACCAGGCGT TTCCCCCTGG AAGCTCCCTC GTGCGCTCTC CTGTTCCGAC CCTGCCGCTT
                                                                           7080
      ACCGGATACC TGTCCGCCTT TCTCCCTTCG GGAAGCGTGG CGCTTTCTCA ATGCTCACGC
                                                                           7140
      TGTAGGTATC TCAGTTCGGT GTAGGTCGTT CGCTCCAAGC TGGGCTGTGT GCACGAACCC
                                                                           7200
```

					OTTOTAL BOTTO	CNACCCCCTA	7260
	CCCGTTCAGC	CCGACCGCTG	CGCCTTATCC	GGTAACTATC	GTCTTGAGTC	ACCOCCOUNT	7320
	AGACACGACT	TATCGCCACT	GGCAGCAGCC	ACTGGTAACA	GGATTAGCAG	AGCGAGGTAT	
	GTAGGCGGTG	CTACAGAGTT	CTTGAAGTGG	TGGCCTAACT	ACGGCTACAC	TAGAAGGACA	7380
	CTATTTCCTA	TCTGCGCTCT	GCTGAAGCCA	GTTACCTTCG	GAAAAAGAGT	TGGTAGCTCT	7440
5	TCATCCGGCA	AACAAACCAC	CGCTGGTAGC	GGTGGTTTTT	TTGTTTGCAA	GCAGCAGATT	7500
5	NOSCGCAGAA	AAAAAGGATC	TCAAGAAGAT	CCTTTGATCT	TTTCTACGGG	GTCTGACGCT	7560
	CACTCGAACG	AAAACTCACG	TTAAGGGATT	TTGGTCATGA	GATTATCAAA	AAGGATCTTC	7620
	ACCTACATCC	ATTAAATTA	AAAATGAAGT	TTTAAATCAA	TCTAAAGTAT	ATATGAGTAA	7680
	ACCIAGAICC	ACAGTTACCA	ATGCTTAATC	AGTGAGGCAC	CTATCTCAGC	GATCTGTCTA	7740
10	ACTIGGICIO	CCATACTTCC	CTGACTCCCC	GTCGTGTAGA	TAACTACGAT	ACGGGAGGGC	7800
10	TITCGITCAT	CCCCACTGC	TGCAATGATA	CCGCGAGACC	CACGCTCACC	GGCTCCAGAT	7860
	TTACCATCIG	GCCCCAGIGC	ACCCCCAAGG	GCCGAGCGCA	GAAGTGGTCC	TGCAACTTTA	7920
	TTATCAGCAA	TANACCAGCC	MA A TOTOTOC	CCCCAACCTA	GAGTAAGTAG	TTCGCCAGTT	7980
	TCCGCCTCCA	TCCAGTCTAT	TAMITGITGC	A CACCCATCC	TCCTCTCACC	CTCGTCGTTT	8040
	AATAGTTTGC	GCAACGTTGT	TGCCATTGCT	ACAGGCATCG	TGGTGTCACG	ATCCCCCATG	8100
15	GGTATGGCTT	CATTCAGCTC	CGGTTCCCAA	CGATCAAGGC	GAGTTACATG	MARCETTOCCCC	8160
	TTGTGCAAAA	AAGCGGTTAG	CTCCTTCGGT	CCTCCGATCG	TTGTCAGAAG	TANGTIGGCC	8220
	GCAGTGTTAT	CACTCATGGT	TATGGCAGCA	CTGCATAATT	CTCTTACTGT	CATGCCATCC	
	GTAAGATGCT	TTTCTGTGAC	TGGTGAGTAC	TCAACCAAGT	CATTCTGAGA	ATAGTGTATG	8280
	CGGCGACCGA	GTTGCTCTTG	CCCGGCGTCA	ATACGGGATA	ATACCGCGCC	ACATAGCAGA	8340
20	ACTTTABAAG	TGCTCATCAT	TGGAAAACGT	TCTTCGGGGC	GAAAACTCTC	AAGGATCTTA	8400
	CCCCTGTTGA	GATCCAGTTC	GATGTAACCC	ACTCGTGCAC	CCAACTGATC	TTCAGCATCT	8460
	TTTTCA	CCAGCGTTTC	TGGGTGAGCA	AAAACAGGAA	GGCAAAATGC	CGCAAAAAAG	8520
	CCDATACCC	CCACACGGAA	ATGTTGAATA	CTCATACTCT	TCCTTTTTCA	ATATTATTGA	8580
	GGAATAAGGG	AGGGTTATTG	TCTCATGAGC	GGATACATAT	TTGAATGTAT	TTAGAAAAAT	8640
25	AGCATITATO	ACCUMENCE	CACATTTCCC	CGAAAAGTGC	CACCTGACGT	С	8691
25	AAACAAATAG	GGGTTCCGCG	CACALITCCC				

(2) INFORMATION FOR SEQ ID NO:10:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 8327 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear
- 35 (ii) MOLECULE TYPE: cDNA

30

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

	01 0001 TCCC	CAGATOTGOT	AGGTGACCTG	AGGCGCGCCG	GCTTCGAATA	GCCAGAGTAA	60
40	GACGGATCGG	TAATTTTATT	TTATTTTATT		AGTTTGGCGC	CGATCTCCCG	120
40	ATCCCCTATG				CGCATAGTTA	AGCCAGTATC	180
		TTGTGTGTTTG				TAAGCTACAA	240
		CTTGACCGAC			TAGGGTTAGG	CGTTTTGCGC	300
	CAAGGCAAGG					AGTTATTAAT	360
4.5	TGCTTCGCGA	TACGGGGTCA				GTTACATAAC	420
45	AGTAATCAAT	•			CCGCCCATTG	ACGTCAATAA	480
	• • • • • • • • • • • • • • • • • • • •	TCCCATAGTA			••••	••	540
	TGACGTATGT				TCATATGCCA		600
						ATGACCTTAT	660
	CTATTGACGT		AAATGGCCCG		CGCTATTACC		720
50		TACTTGGCAG			CTCACGGGGA	-	780
	GGTTTTGGCA				AAATCAACGG		840
	TCCACCCCAT		GGGAGTTTGT				900
	aatgtcgtaa				TAGGCGTGTA	CTTATCGAAA	960
	TCTATATAAG				TGCTTACTGG	•	1020
55	TTAATACGAC		GAGACCCAAG		ATTTAAATTG	ATATCTCCTT	1020
	AGGTCTCGAG	TCTCTAGATA	ACCGGTCAAT				
	CACCATGGAG	TTGTGGTTAA	GCTTGGTCCT	TCCTTGTCCT	TGTTTTAAAA		1140
	GTGAAGTGAA	TCTGGTGGAG	TCTGGGGGAG		-	TCCCTGAAAG	1200
	TCTCCTGTGT	AACCTCTGGA	TTCACTTTCA	GTGACTATTA	CATGTATTGG	GTTCGCCAGA	1260

```
CTCCAGAGAA GAGGCTGGAG TGGGTCGCAT ACATTAGTCA AGGTGGTGAT ATAACCGACT
                                                                           1320
      ATCCAGACAC TGTAAAGGGT CGATTCACCA TCTCCAGAGA CAATGCCAAG AACACCCTGT
                                                                           1380
      ACCTGCAAAT GAGCCGTCTG AAGTCTGAGG ACACAGCCAT GTATTACTGT GCAAGAGGCC
                                                                           1440
      TGGACGACGG GGCCTGGTTT GCTTACTGGG GCCAAGGGAC TCTGGTCACG GTCTCTGTAG
      CTAGCACCAA GGGCCCATCG GTCTTCCCCC TGGCACCCTC CTCCAAGAGC ACCTCTGGGG
                                                                           1560
      GCACAGCGGC CCTGGGCTGC CTGGTCAAGG ACTACTTCCC CGAACCGGTG ACGGTGTCGT
                                                                           1620
      GGAACTCAGG CGCCCTGACC AGCGGCGTGC ACACCTTCCC GGCTGTCCTA CAGTCCTCAG
                                                                           1680
      GACTCTACTC CCTCAGCAGC GTGGTCACCG TGCCCTCCAG CAGCTTGGGC ACCCAGACCT
                                                                           1740
      ACATCTGCAA CGTGAATCAC AAGCCCAGCA ACACCAAGGT GGACAAGAAA GTTGGTGAGA
                                                                           1800
10
      GGCCAGCACA GGGAGGGAGG GTGTCTGCTG GAAGCCAGGC TCAGCGCTCC TGCCTGGACG
                                                                           1860
      CATCCCGGCT ATGCAGCCCC AGTCCAGGGC AGCAAGGCAG GCCCCGTCTG CCTCTTCACC
                                                                           1920
      CGGAGGCCTC TGCCCGCCCC ACTCATGCTC AGGGAGAGGG TCTTCTGGCT TTTTCCCCAG
                                                                           1980
      GCTCTGGGCA GGCACAGGCT AGGTGCCCCT AACCCAGGCC CTGCACACAA AGGGGCAGGT
                                                                           2040
      GCTGGGCTCA GACCTGCCAA GAGCCATATC CGGGAGGACC CTGCCCCTGA CCTAAGCCCA
                                                                           2100
15
      CCCCAAAGGC CAAACTCTCC ACTCCCTCAG CTCGGACACC TTCTCTCCTC CCAGATTCCA
                                                                           2160
      GTAACTCCCA ATCTTCTCTC TGCAGAGCCC AAATCTTGTG ACAAAACTCA CACATGCCCA
                                                                           2220
      CCGTGCCCAG GTAAGCCAGC CCAGGCCTCG CCCTCCAGCT CAAGGCGGGA CAGGTGCCCT
                                                                           2280
      AGAGTAGCCT GCATCCAGGG ACACACCACG TGGGTACCAA CATGTCCGGA GCCACATGGA
                                                                           2340
      CAGAGGCCGG CTCGGCCCAC CCTCTGCCCT GAGAGTGACC GCTGTACCAA CCTCTGTCCC
                                                                           2400
20
      TACAGGGCAG CCCCGAGAAC CACAGGTGTA CACCCTGCCC CCATCCCGGG ATGAGCTGAC
                                                                           2460
      CAAGAACCAG GTCAGCCTGA CCTGCCTGGT CAAAGGCTTC TATCCCAGCG ACATCGCCGT
      GGAGTGGGAG AGCAATGGGC AGCCGGAGAA CAACTACAAG ACCACGCCTC CCGTGCTGGA
                                                                           2580
      CTCCGACGGC TCCTTCTTCC TCTACAGCAA GCTCACCGTG GACAAGAGCA GGTGGCAGCA
                                                                           2640
      GGGGAACGTC TTCTCATGCT CCGTGATGCA TGAGGCTCTG CACAACCACT ACACGCAGAA
                                                                           2700
25
      GAGCCTCTCC CTGTCTCCGG GTAAATGAGT GCGACGGCCG GCAAGCCCCC GCTCCCCGGG
                                                                           2760
      CTCTCGCGGT CGCACGAGGA TGCTTGGCAC GTACCCCCTG TACATACTTC CCGGGCGCCC
                                                                           2820
      AGCATGGAAA TAAAGCACCC AGCGCTGCCC TGGGCCCCTG CGAGACTGTG ATGGTTCTTT
      CCACGGGTCA GGCCGAGTCT GAGGCCTGAG TGGCATGAGG GAGGCAGAGC GGGTCCCACT
                                                                           2940
      GTCCCCACAC TGGCCCAGGC TGTGCAGGTG TGCCTGGGCC CCCTAGGGTG GGGCTCAGCC
30
      AGGGGCTGCC CTCGGCAGGG TGGGGGATTT GCCAGCGTGG CCCTCCCTCC AGCAGCACCT
                                                                           3060
      GCCCTGGGCT GGGCCACGGG AAGCCCTAGG AGCCCCTGGG GACAGACACA CAGCCCCTGC
                                                                           3120
      CTCTGTAGGA GACTGTCCTG TTCTGTGAGC GCCCCTGTCC TCCCGACCTC CATGCCCACT
                                                                           3180
      CGGGGGCATG CCTAGTCCAT GTGCGTAGGG ACAGGCCCTC CCTCACCCAT CTACCCCCAC
                                                                           3240
      GGCACTAACC CCTGGCTGCC CTGCCCAGCC TCGCACCCGC ATGGGGACAC AACCGACTCC
                                                                           3300
35
      GGGGACATGC ACTCTCGGGC CCTGTGGAGG GACTGGTGCA GATGCCCACA CACACACTCA
                                                                           3360
      GCCCAGACCC GTTCAACAAA CCCCGCACTG AGGTTGGCCG GCCACACGGC CACCACACAC
      ACACGTGCAC GCCTCACACA CGGAGCCTCA CCCGGGCGAA CTGCACAGGCA CCCAGACCAG
      AGCAAGGTCC TCGCACACGT GAACACTCCT CGGACACAGG CCCCCACGAG CCCCCACGCGG
                                                                           3540
      CACCTCAAGG CCCACGAGCC TCTCGGCAGC TTCTCCACAT GCTGACCTGC TCAGACAAAC
40
      CCAGCCCTCC TCTCACAAGG GTGCCCCTGC AGCCGCCACA CACACACAGG GGATCACACA
                                                                           3660
      CCACGTCACG TCCCTGGCCC TGGCCCACTT CCCAGTGCCG CCCTTCCCTG CAGGACGGAT
      CAGCCTCGAC TGTGCCTTCT AGTTGCCAGC CATCTGTTGT TTGCCCCTCC CCCGTGCCTT
                                                                           3780
      CCTTGACCCT GGAAGGTGCC ACTCCCACTG TCCTTTCCTA ATAAAATGAG GAAATTGCAT
                                                                           3840
      CGCATTGTCT GAGTAGGTGT CATTCTATTC TGGGGGGTGG GGTGGGGCAG GACAGCAAGG
45
      GGGAGGATTG GGAAGACAAT AGCAGGCATG CTGGGGATGC GGTGGGCTCT ATGGCTTCTG
                                                                           3960
      AGGCGGAAAG AACCAGCTGG GGCTCTAGGG GGTATCCCCA CGCGCCCTGT AGCGGCGCAT
                                                                           4020
      TAAGCGCGGC GGGTGTGGTG GTTACGCGCA GCGTGACCGC TACACTTGCC AGCGCCCTAG
      CGCCCGCTCC TTTCGCTTTC TTCCCTTCCT TTCTCGCCAC GTTCGCCGGG CCTCTCAAAA
                                                                           4140
      AAGGGAAAAA AAGCATGCAT CTCAATTAGT CAGCAACCAT AGTCCCGCCC CTAACTCCGC
                                                                           4200
50
      CCATCCCGCC CCTAACTCCG CCCAGTTCCG CCCATTCTCC GCCCCATGGC TGACTAATTT
                                                                           4260
      TTTTTATTTA TGCAGAGGCC GAGGCCGCCT CGGCCTCTGA GCTATTCCAG AAGTAGTGAG
                                                                           4320
      GAGGCTTTTT TGGAGGCCTA GGCTTTTGCA AAAAGCTTGG ACAGCTCAGG GCTGCGATTT
                                                                           4380
      CGCGCCAAAC TTGACGGCAA TCCTAGCGTG AAGGCTGGTA GGATTTTATC CCCGCTGCCA
                                                                           4440
      TCATGGTTCG ACCATTGAAC TGCATCGTCG CCGTGTCCCA AAATATGGGG ATTGGCAAGA
                                                                           4500
55
      ACGGAGACCT ACCCTGGCCT CCGCTCAGGA ACGAGTTCAA GTACTTCCAA AGAATGACCA
                                                                           456D
      CAACCTCTTC AGTGGAAGGT AAACAGAATC TGGTGATTAT GGGTAGGAAA ACCTGGTTCT
                                                                           4620
      CCATTCCTGA GAAGAATCGA CCTTTAAAGG ACAGAATTAA TATAGTTCTC AGTAGAGAAC
                                                                           4680
      TCAAAGAACC ACCACGAGGA GCTCATTTTC TTGCCAAAAG TTTGGATGAT GCCTTAAGAC
                                                                           4740
      TTATTGAACA ACCGGAATTG GCAAGTAAAG TAGACATGGT TTGGATAGTC GGAGGCAGTT
```

							4000
	CTGTTTACCA	GGAAGCCATG	AATCAACCAG	GCCACCTTAG	ACTCTTTGTG	ACAAGGATCA	4860
	TGCAGGAATT	TGAAAGTGAC	ACGTTTTTCC	CAGAAATTGA	TTTGGGGAAA	TATAAACTTC	4920
	TCCCAGAATA	CCCAGGCGTC	CTCTCTGAGG	TCCAGGAGGA	AAAAGGCATC	AAGTATAAGT	4980
	TTGAAGTCTA	CGAGAAGAAA	GACTAACAGG	AAGATGCTTT	CAAGTTCTCT	GCTCCCCTCC	5040
5	TAAAGCTATG	CATTTTTATA	AGACCATGGG	ACTITIGCTG	GCTTTAGATC	TCTTTGTGAA	5100
	GGAACCTTAC	TTCTGTGGTG	TGACATAATT	GGACAAACTA	CCTACAGAGA	TTTAAAGCTC	5160
	TAAGGTAAAT	TTTTAAAATT	TAAGTGTATA	ATGTGTTAAA	CTACTGATTC	TAATTGTTTG	5220
	TGTATTTTAG	ATTCCAACCT	ATGGAACTGA	TGAATGGGAG	CAGTGGTGGA	ATGCCTTTAA	5280
	TGAGGAAAAC	CTGTTTTGCT	CAGAAGAAAT	GCCATCTAGT	GATGATGAGG	CTACTGCTGA	5340
10	CTCTCAACAT	TCTACTCCTC	CAAAAAAGAA	GAGAAAGGTA	GAAGACCCCA	AGGACTTTCC	5400
	TTCAGAATTG	CTAAGTTTTT	TGAGTCATGC	TGTGTTTAGT	AATAGAACTC	TTGCTTGCTT	5460
	TGCTATTTAC	ACCACAAAGG	AAAAAGCTGC	ACTGCTATAC	AAGAAAATTA	TGGAAAAATA	5520
	TTCTGTAACC	TTTATAAGTA	GGCATAACAG	TTATAATCAT	AACATACTGT	TTTTTCTTAC	5580
	TCCACACAGG	CATAGAGTGT	CTGCTATTAA	TAACTATGCT	CAAAAATTGT	GTACCTTTAG	5640
15	CTTTTTAATT	TGTAAAGGGG	TTAATAAGGA	ATATTTGATG	TATAGTGCCT	TGACTAGAGA	5700
	TCATAATCAG	CCATACCACA	TTTGTAGAGG	TTTTACTTGC	TTTAAAAAAC	CTCCCACACC	5760
	TCCCCCTGAA	CCTGAAACAT	AAAATGAATG	CAATTGTTGT	TGTTAACTTG	TTTATTGCAG	5820
	CTTATAATGG	TTACAAATAA	AGCAATAGCA	TCACAAATTT	CACAAATAAA	GCATTTTTTT	5880
	CACTGCATTC	TAGTTGTGGT	TTGTCCAAAC	TCATCAATGT	ATCTTATCAT	GTCTGGATCG	5940
20	GCTGGATGAT	CCTCCAGCGC	GGGGATCTCA	TGCTGGAGTT	CTTCGCCCAC	CCCAACTTGT	6000
	TTATTGCAGC	TTATAATGGT	TACAAATAAA	GCAATAGCAT	CACAAATTTC	ACAAATAAAG	6060
	CATHITUTIC	ACTGCATTCT	AGTTGTGGTT	TGTCCAAACT	CATCAATGTA	TCTTATCATG	6120
	TCTGTATACC	GTCGACCTCT	AGCTAGAGCT	TGGCGTAATC	ATGGTCATAG	CTGTTTCCTG	6180
-	TCTCAAATTG	TTATCCGCTC	ACAATTCCAC	ACAACATACG	AGCCGGAAGC	ATAAAGTGTA	6240
25	AAGCCTGGGG	TGCCTAATGA	GTGAGCTAAC	TCACATTAAT	TGCGTTGCGC	TCACTGCCCG	6300
20	CTTTCCAGTC	GGGAAACCTG	TCGTGCCAGC	TGCATTAATG	AATCGGCCAA	CGCGCGGGGA	6360
	GAGGCGGTTT	GCGTATTGGG	CGCTCTTCCG	CTTCCTCGCT	CACTGACTCG	CTGCGCTCGG	6420
	TCGTTCGGCT	GCGGCGAGCG	GTATCAGCTC	ACTCAAAGGC	GGTAATACGG	TTATCCACAG	6480
	AATCAGGGGA	TAACGCAGGA	AAGAACATGT	GAGCAAAAGG	CCAGCAAAAG	GCCAGGAACC	6540
30	GTAAAAAGGC	CGCGTTGCTG	GCGTTTTTCC	ATAGGCTCCG	CCCCCTGAC	GAGCATCACA	6600
50	AAAATCGACG	CTCAAGTCAG	AGGTGGCGAA	ACCCGACAGG	ACTATAAAGA	TACCAGGCGT	6660
	TTCCCCCTCC	AAGCTCCCTC	GTGCGCTCTC	CTGTTCCGAC	CCTGCCGCTT	ACCGGATACC	6720
	TOTOGGGGTT	TCTCCCTTCG	GGAAGCGTGG	CGCTTTCTCA	ATGCTCACGC	TGTAGGTATC	6780
	TCACTTCCCT	GTAGGTCGTT	CGCTCCAAGC	TGGGCTGTGT	GCACGAACCC	CCCGTTCAGC	6840
35	CCCACCCCTG	CGCCTTATCC	GGTAACTATC	GTCTTGAGTC	CAACCCGGTA	AGACACGACT	6900
33	TATCCCCACT	GGCAGCAGCC	ACTGGTAACA	GGATTAGCAG	AGCGAGGTAT	GTAGGCGGTG	6960
		CTTGAAGTGG					7020
						TGATCCGGCA	7080
						ACGCGCAGAA	7140
40	ANANACCATC	TCAAGAAGAT	CCTTTGATCT	TTTCTACGGG	GTCTGACGCT	CAGTGGAACG	7200
40	ANAROGRIC	TTAAGGGATT	TTGGTCATGA	CATTATCAAA	AAGGATCTTC	ACCTAGATCC	7260
		AAAATGAAGT					7320
	ACACTTACCA	ATGCTTAATC	AGTGAGGCAC	CTATCTCAGC	GATCTGTCTA	TTTCGTTCAT	7380
						TTACCATCTG	7440
45						TTATCAGCAA	7500
43						TCCGCCTCCA	7560
		TAATTGTTGC					7620
						GGTATGGCTT	7680
						TTGTGCAAAA	7740
50						GCAGTGTTAT	
50	CACTCATCCT	TATCCCACCA	CTCCOATCO	CTCTTACTCT	CATGCCATCC	GTAAGATGCT	7860
						CGGCGACCGA	7920
							7980
						ACTTTAAAAG CCGCTGTTGA	8040
55							8100
رر						TTTACTTTCA	8160
						GGAATAAGGG	8220
						AGCATTTATC	
						AAACAAATAG	8280
	GGGTTCCGCG	CACATTTCCC	CGAAAAGTGC	CACCIGACGT	CCBRAAG	•	8327

PCT/US97/13562

5

(2) INFORMATION FOR SEQ ID NO:11:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 8897 base pairs

(B) TYPE: nucleic acid
(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

10 (ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

```
GGTACCAATT TAAATTGATA TCTCCTTAGG TCTCGAGCAC CATGAAGTTG CCTGTTAGGC
     TGTTGGTGCT GATGTTCTGG ATTCCTGCTT CCAGCAGTGA TGTTTTGATG ACCCAAATTC
     CAGTCTCCCT GCCTGTCAGT CTTGGAGATC AAGCGTCCAT CTCTTGCAGA TCTAGTCAGA
     TCATTGTACA TAATAATGGC AACACCTATT TAGAATGGTA CCTGCAGAAA CCAGGCCAGT
                                                                         240
     CTCCACAGCT CCTGATCTAC AAAGTTTCCA ACCGATTTTC TGGGGTCCCA GACAGGTTCA
     GCGGCAGTGG ATCAGGGACA GATTTCACAC TCAAGATCAG CAGAGTGGAG GCTGAGGATC
     TGGGAGTTTA TTACTGCTTT CAAGGTTCAC ATGTTCCATT CACGTTCGGC TCGGGGACAA
                                                                         420
20
     AGTTGGAAAT AAAACGTAAG TCTCGAGTCT CTAGATAACC GGTCAATCGA TTGGAATTCT
     ANACTOTGAG GGGGTCGGAT GACGTGGCCA TTCTTTGCCT ANAGCATTGA GTTTACTGCA
                                                                         540
     AGGTCAGAAA AGCATGCAAA GCCCTCAGAA TGGCTGCAAA GAGCTCCAAC AAAACAATTT
     AGAACTTTAT TAAGGAATAG GGGGAAGCTA GGAAGAAACT CAAAACATCA AGATTTTAAA
                                                                          720
     TACGCTTCTT GGTCTCCTTG CTATAATTAT CTGGGATAAG CATGCTGTTT TCTGTCTGTC
25
     CCTAACATGC CCTTATCCGC AAACAACACA CCCAAGGGCA GAACTTTGTT ACTTAAACAC
                                                                          780
     CATCCTGTTT GCTTCTTTCC TCAGGAACTG TGGCTGCACC ATCTGTCTTC ATCTTCCCGC
                                                                          840
      CATCTGATGA GCAGTTGAAA TCTGGAACTG CCTCTGTTGT GTGCCTGCTG AATAACTTCT
                                                                         900
     ATCCCAGAGA GGCCAAAGTA CAGTGGAAGG TGGATAACGC CCTCCAATCG GGTAACTCCC
     AGGAGAGTGT CACAGAGCAG GAGAGCAAGG ACAGCACCTA CAGCCTCAGC AGCACCCTGA
                                                                         1020
      CGCTGAGCAA AGCAGACTAC GAGAAACACA AAGTCTACGC CTGCGAAGTC ACCCATCAGG
     GCCTGAGCTC GCCCGTCACA AAGAGCTTCA ACAGGGGAGA GTGTTAGAGG GAGAAGTGCC
                                                                         1140
      CCCACCTGCT CCTCAGTTCC AGCCTGACCC CCTCCCATCC TTTGGCCTCT GACCCTTTTT
      CCACAGGGGA CCTACCCCTA TTGCGGTCCT CCAGCTCATC TTTCACCTCA CCCCCCTCCT
      CCTCCTTGGC TTTAATTATG CTAATGTTGG AGGAGAATGA ATAAATAAAG TGAATCTTTG
35
      CACCTGTGGT TTCTCTCTT CCTCATTTAA TAATTATTAT CTGTTGTTTT ACCAACTACT
                                                                         1380
      CAATTTCTCT TATAAGGGAC TAAATATGTA GTCATCCTAA GGCACGTAAC CATTTATAAA
                                                                         1440
      AATCATCCTT CATTCTATTT TACCCTATCA TCCTCTGCAA GACAGTCCTC CCTCAAACCC
                                                                         1500
      ACAAGCCTTC TGTCCTCACA GTCCCCTGGG CCATGGTAGG AGAGACTTGC TTCCTTGTTT
      TCCCCTCCTC AGCAAGCCCT CATAGTCCTT TTTAAGGGTG ACAGGTCTTA CAGTCATATA
      TCCTTTGATT CAATTCCCTG AGAATCAACC AAAGCAAATT TTTCAAAAGA AGAAACCTGC
      TATAAAGAGA ATCATTCATT GCAACATGAT ATAAAATAAC AACACAATAA AAGCAATTAA
      ATAAACAAAC AATAGGGAAA TGTTTAAGTT CATCATGGTA CTTAGACTTA ATGGAATGTC
                                                                         1800
      ATGCCTTATT TACATTTTTA AACAGGTACT GAGGGACTCC TGTCTGCCAA GGGCCGTATT
                                                                         1860
      GAGTACTITC CACAACCTAA TITAATCCAC ACTATACTGT GAGATTAAAA ACATTCATTA
45
      ARATGTTGCA AAGGTTCTAT AAAGCTGAGA GACAAATATA TTCTATAACT CAGCAATCCC
                                                                         2040
      ACTICIAGAT GACTGAGTGT CCCCACCCAC CAAAAAACTA TGCAAGAATG TTCAAAGCAG
      CTTTATTTAC AAAAGCCAAA AATTGGAAAT AGCCCGATTG TCCAACAATA GAATGAGTTA
                                                                         2100
      TTAAACTGTG GTATGTTTAT ACATTAGAAT ACCCAATGAG GAGAATTAAC AAGCTACAAC
                                                                         2160
                                                                         2220
      TATACCTACT CACACAGATG AATCTCATAA AAATAATGTT ACATAAGAGA AACTCAATGC
50
      AAAAGATATG TTCTGTATGT TTTCATCCAT ATAAAGTTCA AAACCAGGTA AAAATAAAGT
                                                                         2280
      TAGAAATTTG GATGGAAATT ACTCTTAGCT GGGGGTGGGC GAGTTAGTGC CTGGGAGAAG
                                                                         2340
      ACAAGAAGGG GCTTCTGGGG TCTTGGTAAT GTTCTGTTCC TCGTGTGGGG TTGTGCAGTT
                                                                         2400
      ATGATCTGTG CACTGTTCTG TATACACATT ATGCTTCAAA ATAACTTCAC ATAAAGAACA
                                                                         2460
      TCTTATACCC AGTTAATAGA TAGAAGAGGA ATAAGTAATA GGTCAAGACC AACGCAGCTG
                                                                         2520
 55
      GTAAGTGGGG GCCTGGGATC AAATAGCTAC CTGCCCTAATC CTGCCCWCTT GAGCCCTGAA
      CATCTGTGCC CTGTTTGGCT AGCTAGGAGC ACACATACAT AGAAATTAAA TGAAACAGAC
                                                                         2700
       CTTCAGCAAG GGGACAGAGG ACAGAATTAA CCTTGCCCAG ACACTGGAAA CCCATGTATG
```

						тсстсаттст	2820
	AACACTCACA	TGTTTGGGAA (GGGGAAGGG	CACATGTAAA	TGAGGACICI	CTTTCTAAGT	2880
		AMACACAMICA I	ついりはつかつかつつつ	TACTCATCCA	TOWNCHONG	CITICIPAGE	2940
		COURT OR CTC 1	かけいりついん ないか	CAGGAGTAAC	TAACACAGCA	ICCCITCCCI	3000
		A CHARGO COMP	かいかい かんしん かんしん こうりん こうりん しんしょう しんしょう しんしょう しんしょう しんしょう しゅうしゅう しゅう	TGTTTTTCTT	TOTAGICAGI	WC 1 GGGLRRIG	3060
5		OR OTHER TRUES.	ሚ አል አርሞ አር እጥ	AAGGAAGCAC	CLICCTIC	IGCCICITON	3120
•		A A AMPAROL A	שמממחדרים מאברים	TTTGGAGGTT	TGAGIAGGG	IGNOUCICUO	3180
	TAATGTCCCT	TCCAATGACA	TGAACTTGCT	CACTCATCCC	TGGGGGCCAA	CACCITCITTCC	3240
		CAMPARICONG	ጥጥልጥርልልጥጥር	TTGCGGCCGC	TIGGINGCII	CACGIGITOG	3300
			ጥአጥጥጥአጥልር	TGTCACCTAA	AIGCINGAGC	ICCCIONICA	3360
10		macammata?	ガーサンこへしかいこくしか	TCTGTTGTTT		CGIGCCIICC	3420
		********	ጥሶሶሶልርማናፕሮ	CTTTCCTAAT	WWWIGHT	Willowinge	3480
		ADD COMOTOR	ביות אוריים איניים	GGGGGTGGGG	TGGGGGCAGGA	CHOCHHOOOG	3540
		220202000	<u> </u>	GGGGATGCGG	IGGGCICIMI	GGCTTCTGAG	3600
		ACT COMCCCC	こうごうごう ダヤウヤン	TATCCCCACG	CCCCTGTMG	COCCOCALIA	3660
15		CHCCCCCCCCC	でなべなべなべなべるほど	GTGACCGCTA	CACTIGCCAG	CACCCINACA	3720
		MACARTTCTT	بالشارات الملحات	CTCGCCACGT	10000000	TCICHARAN	3780
		TOTAL PROPERTY.	ሲጎፐጋፈጥጥል	GCAACCATAG	TUCCGCCCCCT	MACICCOCCC	3840
			ペルグサヤイン	CATTCTCCGC	CCCATGGCTG	WCINUTIAN	3900
		CACACCCCCA	CCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCC	GCCTCTGAGC	TATTCCAGAA	GINGIGNGGN	3960
20		ON COCCENCO	TAA CONTINUE TO A A A A A A A A A A A A A A A A A A	AAGCTTGGAC	AGCTCAGGGC	IGCGWIIICG	
20		OT COCCO ATC	CTACCCTGAA	GGCTGGTAGG	ATTTTATCCC	CGCIGCCMIC	4020 4080
		OR MONOR & COTO:	CATCCTCCC	GTGTCCCAAA	ATATGGGGAT	IGGCIMONIC	
		acmorace correct	CCTCAGGAAC	GAGTTCAAGT	ACTICCAAAG	WATGWCCHCU	4140 4200
		MACAN NOOTH A	አሮአርአአጥሮፕር	GTGATTATGG	CIAGGAAAA	CIGGILETEE	4260
25		ACARTCCACC	TTTAAAGGAC	AGAATTAATA	TAGTTCTCAG	INGMOMNCIC	4320
		CROCKCCACC		GCCAAAAGTT	TGGATGATGC	CIIMMONCII	4380
		CCCNNTTCCC	AAGTAAAGTA	GACATGGTTT	GGATAGTCGG	Medicagnici	4440
		A A COCCATCA A	TODACCAGG	CACCITAGAC	TCTTTGTGAC	MAGGAIGHIG	4500
		BBBCTCBCBC	COCOTTANTO	GAAATTGATT	TGGGGAAATA	IMMCIICIC	4560
30		CACCCCTCCT	CTCTCAGGTC	'CAGGAGGAAA	AAGGCATCAA	GINIMAGILI	4620
-		3033033303	CTAACAGGAA	GATGCTTTCA	AGTTCTCTGC	LICCUCIO	4680
	AAGCTATGCA	TTTTTATAAG	ACCATGGGAC	TTTTGCTGGC	TITAGATCIC	TTTGTGAAGG	4740
	AACCTTACTT	CTGTGGTGTG	ACATAATTGG	ACAAACTACC	TACAGAGATI	TAAAGCTCTA	4800
	AGGTAAATAT	ATTTTTAAAA	AGTGTATAAT	GTGTTAAACT	ACTUALICIA	ATTGTTTGTG	4860
35	TATTTTAGAT	TCCAACCTAT	GGAACTGATG	AATGGGAGCA	mcarcaccc	DTAATTTOOD T	4920
	AGGAAAACCT	GTTTTGCTCA	GAAGAAATG	CATCTAGTGA	ACACCCCAAC	ACTGCTGACT	4980
	CTCAACATTC	TACTCCTCCA	AAAAAGAAGA	GAAAGGTAGA	MONCOCON.	GACTTTCCTT	5040
	CAGAATTGCT	AAGTTTTTTG	AGTCATGCTC	TGTTTAGTAA	TAGAACICI.	GCTTGCTTTG	5100
	CTATTTACAC	: CACAAAGGAA	AAAGCTGCAG	TGCTATACAA	CAMMATIA	GAAAAATATT	5160
40	CTGTAACCTT	TATAAGTAGG	CATAACAGT	r ATAATCATAA	CAIACIGII	TTTCTTACTC	5220
	CACACAGGCA	TAGAGTGTCT	GCTATTAAT	A ACTATECTCA	MARKITOTO:	ACCTTTAGCT	5280
	TTTTAATTTG	TAAAGGGGTT	AATAAGGAA	r ATTIGATGIA	I IMOIOCCII	ACTAGAGATC	5340
	ATAATCAGCC	: ATACCACATI	TGTAGAGGT	r Tracrigui	TAMAMANCE TAMAMANCE	T CCCACACCTC	5400
	CCCCTGAACC	TGAAACATAA	AATGAATGC	A ATTGTTGTTG	CANACITOL	TATTGCAGCT	5460
45	TATAATGGTT	ACAAATAAAG	CAATAGCAT	C ACAAATTIC	CAAATAAAC	C ATTTTTTCA	5520
	CTGCATTCT	A GTTGTGGTTT	GTCCAAACT	C ATCAALGIA	TOGOCOACO	T CTGGATCGGC	5580
	TGGATGATC	TCCAGCGCGC	GGATCTCAT	G CIGGAGIIC	CALATTTCA	C CAACTTGTTT C AAATAAAGCA	5640
	ATTGCAGCT	r ataatggtta	CAAATAAAG	C MAIAGUAIU	TCARTGTAT	C AAATAAAGCA C TTATCATGTC	5700
	TTTTTTTCA	C TGCATTCTAC	TIGIGGITI	C CCCTAATCAC	CGTCATAGC	C TTATCATGTC T GTTTCCTGTG	5760
50	TGTATACCG'	r CGACCTCTAC	CIAGAGCII	G GCGIMAICA	CCCCARCCA	T AAAGTGTAAA	5820
	TGAAATTGT	I ATCCGCTCAC	AATTCCACA	C VOTALVEN	CCCCARGOCA CCTTCCCCCT	T AAAGTGTAAA	5880
	GCCTGGGGT	G CCTAATGAG	GAGCTAACT	C WCWIIWWIII	TCGGTCVATC	C ACTGCCCGCT	5940
	TTCCAGTCG	G GAAACCTGT	GIGCCAGCT	A CHITANIAN	הדנושיכיהרנור מושטיביהורנור	G CGCGGGGAGA	6000
	GGCGGTTTG	C GTATTGGGC	CTCTTCCGC	C TONARCOCC	TENTINGE	T GCGCTCGGTC	6060
55	GTTCGGCTG	C GGCGAGCGG	r ATCAGCTCA	C ICMMAGGGG	C DECEMBER OF	T ATCCACAGAA C CAGGAACCGT	6120
	TCAGGGGAT	A ACGCAGGAA	GAACATGTG	T ACCUMENTS	C CCCCTGACG	CAGGAACCGT A GCATCACAAA	6180
	AAAAAGGCC	G CGTTGCTGG	GITTITCCA	T WORLIGGE	C TATABAGAT	A CCAGGCGTTT	6240
	AATCGACGC	T CAAGTCAGA	GIGGCGAAA	TO COMMINGA	C TGCCGCTT	A CCAGGCGTTT AC CGGATACCTG	
	CCCCTGGA	A GCTCCCTCG	r GCGCTCTCC	.1 GIICCOACC	- 10000011		

```
TCCGCCTTTC TCCCTTCGGG AAGCGTGGCG CTTTCTCAAT GCTCACGCTG TAGGTATCTC
      AGTTCGGTGT AGGTCGTTCG CTCCAAGCTG GGCTGTGTGC ACGAACCCCC CGTTCAGCCC
                                                                          6420
      GACCGCTGCG CCTTATCCGG TAACTATCGT CTTGAGTCCA ACCCGGTAAG ACACGACTTA
                                                                          6480
      TCGCCACTGG CAGCAGCCAC TGGTAACAGG ATTAGCAGAG CGAGGTATGT AGGCGGTGCT
     ACAGAGTTCT TGAAGTGGTG GCCTAACTAC GGCTACACTA GAAGGACAGT ATTTGGTATC
                                                                          6600
      TGCGCTCTGC TGAAGCCAGT TACCTTCGGA AAAAGAGTTG GTAGCTCTTG ATCCGGCAAA
                                                                          6660
      CAAACCACCG CTGGTAGCGG TGGTTTTTTT GTTTGCAAGC AGCAGATTAC GCGCAGAAAA
      AAAGGATCTC AAGAAGATCC TTTGATCTTT TCTACGGGGT CTGACGCTCA GTGGAACGAA
                                                                          6780
      AACTCACGTT AAGGGATTTT GGTCATGAGA TTATCAAAAA GGATCTTCAC CTAGATCCTT
                                                                          6840
10
      TTAAATTAAA AATGAAGTTT TAAATCAATC TAAAGTATAT ATGAGTAAAC TTGGTCTGAC
                                                                          6900
      AGTTACCAAT GCTTAATCAG TGAGGCACCT ATCTCAGCGA TCTGTCTATT TCGTTCATCC
                                                                          6960
      ATAGTTGCCT GACTCCCCGT CGTGTAGATA ACTACGATAC GGGAGGGCTT ACCATCTGGC
                                                                          7020
      CCCAGTGCTG CAATGATACC GCGAGACCCA CGCTCACCGG CTCCAGATTT ATCAGCAATA
                                                                           7080
      AACCAGCCAG CCGGAAGGGC CGAGCGCAGA AGTGGTCCTG CAACTTTATC CGCCTCCATC
                                                                           7140
      CAGTCTATTA ATTGTTGCCG GGAAGCTAGA GTAAGTAGTT CGCCAGTTAA TAGTTTGCGC
15
                                                                          7200
      AACGTTGTTG CCATTGCTAC AGGCATCGTG GTGTCACGCT CGTCGTTTGG TATGGCTTCA
                                                                           7260
      TTCAGCTCCG GTTCCCAACG ATCAAGGCGA GTTACATGAT CCCCCATGTT GTGCAAAAAA
                                                                           7320
      GCGGTTAGCT CCTTCGGTCC TCCGATCGTT GTCAGAAGTA AGTTGGCCGC AGTGTTATCA
                                                                           7380
      CTCATGGTTA TGGCAGCACT GCATAATTCT CTTACTGTCA TGCCATCCGT AAGATGCTTT
                                                                           7440
20
      TCTGTGACTG GTGAGTACTC AACCAAGTCA TTCTGAGAAT AGTGTATGCG GCGACCGAGT
                                                                           7500
                                                                           7560
      TGCTCTTGCC CGGCGTCAAT ACGGGATAAT ACCGCGCCAC ATAGCAGAAC TTTAAAAGTG
      CTCATCATTG GAAAACGTTC TTCGGGGCGA AAACTCTCAA GGATCTTACC GCTGTTGAGA
                                                                           7620
      TCCAGTTCGA TGTAACCCAC TCGTGCACCC AACTGATCTT CAGCATCTTT TACTTTCACC
                                                                           7680
      AGCGTTTCTG GGTGAGCAAA AACAGGAAGG CAAAATGCCG CAAAAAAGGG AATAAGGGCG
                                                                           7740
      ACACGGAAAT GTTGAATACT CATACTCTTC CTTTTTCAAT ATTATTGAAG CATTTATCAG
25
                                                                           7800
      GGTTATTGTC TCATGAGCGG ATACATATTT GAATGTATTT AGAAAAATAA ACAAATAGGG
      GTTCCGCGCA CATTTCCCCG AAAAGTGCCA CCTGACGTCG ACGGATCGGG AGATCTGCTA
                                                                           7920
      GCCCGGGTGA CCTGAGGCGC GCCGGCTTCG AATAGCCAGA GTAACCTTTT TTTTTAATTT
                                                                           7980
      TATTTTATTT TATTTTTGAG ATGGAGTTTG GCGCCGATCT CCCGATCCCC TATGGTCGAC
                                                                           8040
      TCTCAGTACA ATCTGCTCTG ATGCCGCATA GTTAAGCCAG TATCTGCTCC CTGCTTGTGT
30
                                                                           8100
      GTTGGAGGTC GCTGAGTAGT GCGCGAGCAA AATTTAAGCT ACAACAAGGC AAGGCTTGAC
                                                                           8160
      CGACAATTGC ATGAAGAATC TGCTTAGGGT TAGGCGTTTT GCGCTGCTTC GCGATGTACG
                                                                           8220
      GGCCAGATAT ACGCGTTGAC ATTGATTATT GACTAGTTAT TAATAGTAAT CAATTACGGG
                                                                           8280
      GTCATTAGTT CATAGCCCAT ATATGGAGTT CCGCGTTACA TAACTTACGG TAAATGGCCC
      GCCTGGCTGA CCGCCCAACG ACCCCCGCCC ATTGACGTCA ATAATGACGT ATGTTCCCAT
35
                                                                           8400
      AGTAACGCCA ATAGGGACTT TCCATTGACG TCAATGGGTG GACTATTTAC GGTAAACTGC
                                                                           B460
      CCACTTGGCA GTACATCAAG TGTATCATAT GCCAAGTACG CCCCCTATTG ACGTCAATGA
      CGGTAAATGG CCCGCCTGGC ATTATGCCCA GTACATGACC TTATGGGACT TTCCTACTTG
                                                                           8580
      GCAGTACATC TACGTATTAG TCATCGCTAT TACCATGGTG ATGCGGTTTT GGCAGTACAT
40
      CAATGGGCGT GGATAGCGGT TTGACTCACG GGGATTTCCA AGTCTCCACC CCATTGACGT
                                                                           8700
      CAATGGGAGT TTGTTTTGGC ACCAAAATCA ACGGGACTTT CCAAAATGTC GTAACAACTC
                                                                           8760
      CGCCCCATTG ACGCAAATGG GCGGTAGGCG TGTACGGTGG GAGGTCTATA TAAGCAGAGC
                                                                           8820
      TCTCTGGCTA ACTAGAGAAC CCACTGCTTA CTGGCTTATC GAAATTAATA CGACTCACTA
                                                                           8880
                                                                           8897
      TAGGGAGACC CAAGCTT
45
                (2) INFORMATION FOR SEQ ID NO:12:
```

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 8321 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

50

55

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:

GGTACCAATT TAAATTGATA TCTCCTTAGG TCTCGAGTCT CTAGATAACC GGTCAATCGA
TTGGAATTCT TGCGGCCGCT TGCTAGCCAC CATGGAGTTG TGGTTAAGCT TGGTCTTCCT 120

					amaan amama	CCCCACCCTT	180
	TGTCCTTGTT	TTAAAAGGTG	TCCAGTGTGA	AGTGCAACTG	GIGGWGICIG	CCTTCACTGA	240
	AGTGCAGCCT	GGAGGGTCCC	TGCGACTTTC	CTGTGCTGCA	TUIGGATICC	TOTONTACAT	300
	CTATTACATG	TATTGGGTTC	GCCAGGCTCC	AGGCAAGGGA	CTGGAGTGGG	TCTCATACAT	360
	TAGTCAAGAT	GGTGATATAA	CCGACTATGC	AGACTCCGTA	AAGGGTCGAT	TCACCATCTC	420
5	CAGAGACAAT	GCAAAGAACA	GCCTGTACCT	GCAAATGAAC	AGCCTGAGGG	ACGAGGACAC	
-		ምእርጥርጥርር ሺ <u>ል</u>	GAGGCCTGGC	GGACGGGGCC	TGGTTTGCTT	ACTGGGGCCA	480
	NOOR OF CITY	CTCACCCTCT	CTTCCGCTAG	CACCAAGGGC	CCATCGGTCT	TUCCUCTUGG	540
		ANCAGCACCT	CTGGGGGGCAC	AGCGGCCCTG	GGCTGCCTGG	TCAAGGACIA	600
	ammacacaca N	CCCCTGACGG	TGTCGTGGAA	CTCAGGCGCC	CTGACCAGCG	GCGTGCACAC	660
10	CONTRACTOR	CTCCTACAGT	CCTCAGGACT	CTACTCCCTC	AGCAGCGTGG	TCACCGIGCC	720
	CONCORCE CO	TTCCCCCACCC	AGACCTACAT	CTGCAACGTG	AATCACAAGC	CCAGCAACAC	780
	as a composition	AACABACTTC	GTGAGAGGCC	AGCACAGGGA	GGGAGGGTGT	CIGCIGGAAG	840
	ana commence	CCCTCCTCCC	TGGACGCATC	CCGGCTATGC	AGCCCCAGTC	CAGGGCAGCA	900
		COTOTOTO	TTCACCCGGA	GGCCTCTGCC	CGCCCCACTC	ATGUTUAGGG	960
15		CTCCCTTTTT	CCCCAGGCTC	TGGGCAGGCA	CAGGCTAGGT	GCCCCTAACC	1020
13	as according C	NCACABAGGG	GCAGGTGCTG	GGCTCAGACC	TGCCAAGAGC	CATATCCGGG	1080
	A GOA GOOTGO	CCCTGACCTA	AGCCCACCCC	AAAGGCCAAA	CTCTCCACTC	CCTCAGCTCG	1140
	CA CA COTTOT	CTCCTCCCAG	ATTCCAGTAA	CTCCCAATCT	TCTCTCTGCA	GAGCCCAAAT	1200
	GACACCTICI	AACTCACACA	TGCCCACCGT	GCCCAGGTAA	GCCAGCCCAG	GCCTCGCCCT	1260
20	CTTGTGACAA	CCCCCACAC	TGCCCTAGAG	TAGCCTGCAT	CCAGGGACAC	ACCACGTGGG	1320
20	CCAGCTCAAG	TCCCCACCCA	CATGGACAGA	GGCCGGCTCG	GCCCACCCTC	TGCCCTGAGA	1380
	TACCAACATG	TRECONSCER	TOTOCOTACA	GGGCAGCCCC	GAGAACCACA	GGTGTACACC	1440
	GTGACCGCTG	TACCARCCIC	CCTCACCAAG	AACCAGGTCA	GCCTGACCTG	CCTGGTCAAA	1500
	CTGCCCCCAT	CCCGGGAIGA	CCCCCTCCAG	TGGGAGAGCA	ATGGGCAGCC	GGAGAACAAC	1560
25	GGCTTCTATC	CCAGCGACAI	COCCOTOGAG	GACGGCTCCT	TCTTCCTCTA	CAGCAAGCTC	1620
25	TACAAGACCA	CGCCTCCCGT	CCACCACCC	AACGTCTTCT	CATGCTCCGT	GATGCATGAG	1680
	ACCGTGGACA	AGAGCAGGIG	CCAGCAGGGG	CTCTCCCTGT	CTCCGGGTAA	ATGAGTGCGA	1740
	GCTCTGCACA	ACCACTACAC	CCAGAAGAGC	CGCGGTCGCA	CGAGGATGCT	TGGCACGTAC	1800
	CGGCCGGCAA	GCCCCGCTC	CCCGGGCTCT	TCC333TC3CA	CCACCCACCG	CTGCCCTGGG	1860
••	CCCCTGTACA	TACTTCCCGG	GCGCCCAGCA	GGGTCAGGCC	CACTCTGAGG	CCTGAGTGGC	1920
30	CCCCTGCGAG	ACTGTGATGG	TTCTTTCCAC	GGGTCAGGCC	CCACCCTCTC	CAGGTGTGCC	1980
	ATGAGGGAGG	CAGAGCGGGT	CCCACTGTCC	CCACACIGGC	CCAGGCTGTG	CAGGTGTGCC	2040
	TGGGCCCCCT	AGGGTGGGC	TCAGCCAGGG	CCTGCCCTCG	OD CCCCA AGC	GGATTTGCCA	2100
	GCGTGGCCCT	CCCTCCAGCA	GCACCTGCCC	TGGGCTGGGC	CACGGGAAGC	CCTAGGAGCC	2160
	CCTGGGGACA	GACACACAGC	CCCTGCCTCT	GTAGGAGACT	GICCIGIICI	GTGAGCGCCC	2220
35	CTGTCCTCCC	GACCTCCATG	CCCACTCGGG	GGCATGCCTA	GICCAIGIGC	GTAGGGACAG	2280
	GCCCTCCCTC	ACCCATCTAC	CCCCACGGCA	CTAACCCCTG	#CCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCC	CCAGCCTCGC	2340
	ACCCGCATGG	GGACACAACC	GACTCCGGGG	ACATGCACTC	TCGGGCCCTG	TGGAGGGACT	2400
	GGTGCAGATG	CCCACACACA	CACTCAGCCC	AGACCCGTTC	AACAAACCCC	GCACTGAGGT	2460
	TGGCCGGCCA	CACGGCCACC	ACACACACAC	: GTGCACGCCT	CACACACGGA	GCCTCACCCG	2520
40	GGCGAACTGC	ACAGCACCCA	GACCAGAGCA	AGGTCCTCGC	ACACGTGAAC	ACTCCTCGGA	2580
	CACAGGCCCC	CACGAGCCCC	ACGCGGCACC	: TCAAGGCCCA	CGAGCCTCTC	GGCAGCTTCT	
	CCACATGCTG	ACCTGCTCAG	ACAAACCCAG	CCCTCCTCTC	ACAAGGGTGC	CCCTGCAGCC	2640
	CCCACACACA	CACAGGGGAT	CACACACCAC	: GTCACGTCCC	TGGCCCTGGC	CCACTTCCCA	2700
	GTGCCGCCCT	TCCCTGCAGG	ACGGATCAG	CTCGACTGTG	CCTTCTAGTT	GCCAGCCATC	2760
45	TOTAL TATE OF THE PARTY OF THE	CCCTCCCCCG	TGCCTTCCTT	r gaccctggaa	GGTGCCACTC	CCACTGTCCT	2820
	TTCCTAATAA	AATGAGGAAA	TTGCATCGC	A TIGICIGAGI	AGGTGTCATT	CTATTCTGGG	2880
	GGGTGGGGTG	GGGCAGGACA	GCAAGGGGG	GGATTGGGAA	GACAATAGC	GGCATGCTGG	2940
	CCATCCCCTC	GGCTCTATGG	CTTCTGAGG	C GGAAAGAACC	AGCTGGGGC	CIAGGGGGIA	3000
	TOTOTATO	CCCTGTAGCG	GCGCATTAA	CGCGGCGGGT	GTGGTGGTT#	CGCGCAGCGT	3060
50	CACCCCTACA	CTTGCCAGCG	CCCTAGCGC	CCCTCCTTTC	GCTTTCTTC	CITCCITICI	3120
	CCCCACCTTC	CCCGGGCCTC	TCAAAAAAG	GAAAAAAAAGC	: ATGCATCTC	ATTAGTCAGC	3180
	እ <i>እርር</i> እጥ እርጥር	CCGCCCCTAR	CTCCGCCCA'	r cccgccccta	ACTCCGCCC	GTTCCGCCCA	3240
	ササビザビビはごご	CATGGCTGAC	TAATTTTTT	r tatttatgca	GAGGCCGAG	CCCCCTCGGC	3300
	COCOTO A COTT	TTCCAGAAGT	ACTGAGGAGG	: CTTTTTTGGA	GGCCTAGGC	r TTTGCAAAAA	3360
55	CCTTCCACACAC	CTCAGGGCTG	CGATTTCGC	G CCAAACTTGA	CGGCAATCC'	r AGCGTGAAGG	3420
J.J	CTCCTACCAT	TTTATCCCCC	: CTGCCATCA	r ggttcgacca	TTGAACTGC	4 TCGTCGCCG1	3480
	CIGGIAGGA	י אייניניניניאיייי	GCAAGAACG	G AGACCTACCO	TGGCCTCCG	TCAGGAACGA	3540
	クサイク カ カ ク カ カ	TTCCAAAGAZ	TGACCACAA	C CTCTTCAGT	GAAGGTAAA	CAGAATCIGGI	3600
	GII CANGIM	r AGGAAACC	GGTTCTCCA	T TCCTGAGAAG	AATCGACCT	TAAAGGACAG	3660
	GALTATGGG	, AGGAMMACC					

	ATTAATAA	GTTCTCAGTA	GAGAACTCAA	AGAACCACCA	CGAGGAGCTC	ATTITCTIGC	3720
	CAAAAGTTTG	GATGATGCCT	TAAGACITAT	TGAACAACCG	GAATTGGCAA	GTAAAGTAGA	3780
	CATGGTTTGG	ATAGTCGGAG	GCAGTTCTGT	TTACCAGGAA	GCCATGAATC	AACCAGGCCA	3840
	CCTTAGACTC	TTTGTGACAA	GGATCATGCA	GGAATTTGAA	AGTGACACGT	TITTCCCAGA	3900
5	AATTGATTTG	GGGAAATATA	AACTTCTCCC	AGAATACCCA	GGCGTCCTCT	CTGAGGTCCA	3960
	GGAGGAAAAA	GGCATCAAGT	ATAAGTITGA	AGTCTACGAG	AAGAAAGACT	AACAGGAAGA	4020
	TGCTTTCAAG	TTCTCTGCTC	CCCTCCTAAA	GCTATGCATT	TTTATAAGAC	CATGGGACTT	4080
	TTGCTGGCTT	TAGATCTCTT	TGTGAAGGAA	CCTTACTTCT	GTGGTGTGAC	ATAATTGGAC	4140
	AAACTACCTA	CAGAGATTTA	AAGCTCTAAG	GTAAATATAA	AATTTTTAAG	TGTATAATGT	4200
10	GTTAAACTAC	TGATTCTAAT	TGTTTGTGTA	TTTTAGATTC	CAACCTATGG	AACTGATGAA	4260
	TGGGAGCAGT	GGTGGAATGC	CTTTAATGAG	GAAAACCTGT	TTTGCTCAGA	AGAAATGCCA	4320
	TCTAGTGATG	ATGAGGCTAC	TGCTGACTCT	CAACATTCTA	CTCCTCCAAA	AAAGAAGAGA	4380
	AACCTAGAAG	ACCCCAAGGA	CTTTCCTTCA	GAATTGCTAA	GTTTTTTGAG	TCATGCTGTG	4440
	TTTACTAATA	GAACTCTTGC	TIGCTITICT	ATTTACACCA	CAAAGGAAAA	AGCTGCACTG	4500
15	TITOTOTO	AAATTATGGA	ΔΑΔΑΤΑΤΤΟΤ	GTAACCTTTA	TAAGTAGGCA	TAACAGTTAT	4560
13	CIMINCAMON	TACTGTTTTT	ずででするでででる	CACAGGCATA	GAGTGTCTGC	TATTAATAAC	4620
	AATCATAACA	AATTGTGTAC	TCITACTCCT	TTAATTTYTA	AACCCCTTAA	TARGGARTAT	4680
	TATGUTCAAA	GTGCCTTGAC	TACACATCAT	AATCACCCAT	ACCACATTTC	TAGAGGTTTT	4740
	TTGATGTATA	AAAAACCTCC	CACACATCAT	VAICAGCCA1	ADDCATABAA	TCAATCCAAT	4800
20	ACTTGCTTTA	AAAAACCTCC	CACACCICCC	TARTCCTTAC	ANNUALANA	ATACCATCAC	4860
20	TGTTGTTGTT	AACTIGITIA	TIGCAGCITA	CONTROL	WANTANAGCA	CCANACTCAC	4920
	AAATTTCACA	AATAAAGCAT	TTTTTTCACT	GCATTCTAGT	TGTGGTTTGT	CCMMCICAL	4980
	CAATGTATCT	TATCATGTCT	GGATCGGCTG	GATGATCCTC	CAGCGCGGGG	ATCTCATGCT	-
	GGAGTTCTTC	GCCCACCCCA	ACTIGITAT	TGCAGCTTAT	AATGGTTACA	AATAAAGCAA	5040
	TAGCATCACA	AATTTCACAA	ATAAAGCATT	TTTTTCACTG	CATTCTAGTT	GIGGTITGIC	5100
25	CAAACTCATC	AATGTATCTT	ATCATGTCTG	TATACCGTCG	ACCTCTAGCT	AGAGCTTGGC	5160
	GTAATCATGG	TCATAGCTGT	TTCCTGTGTG	AAATTGTTAT	CCGCTCACAA	TTCCACACAA	5220
	CATACGAGCC	GGAAGCATAA	AGTGTAAAGC	CTGGGGTGCC	TAATGAGTGA	GCTAACTCAC	5280
	ATTAATTGCG	TTGCGCTCAC	TGCCCGCTTT	CCAGTCGGGA	AACCTGTCGT	GCCAGCTGCA	5340
	TTAATGAATC	GGCCAACGCG	CGGGGAGAGG	CGGTTTGCGT	ATTGGGCGCT	CTTCCGCTTC	5400
30	CTCGCTCACT	GACTCGCTGC	GCTCGGTCGT	TCGGCTGCGG	CGAGCGGTAT	CAGCTCACTC	5460
	AAAGGCGGTA	ATACGGTTAT	CCACAGAATC	AGGGGATAAC	GCAGGAAAGA	ACATGTGAGC	5520
	AAAAGGCCAG	CAAAAGGCCA	GGAACCGTAA	AAAGGCCGCG	TTGCTGGCGT	TTTTCCATAG	5580
	GCTCCGCCCC	CCTGACGAGC	ATCACAAAAA	TCGACGCTCA	AGTCAGAGGT	GGCGAAACCC	5640
	GACAGGACTA	TAAAGATACC	AGGCGTTTCC	CCCTGGAAGC	TCCCTCGTGC	GCTCTCCTGT	5700
35	TCCGACCCTG	CCGCTTACCG	GATACCTGTC	CGCCTTTCTC	CCTTCGGGAA	GCGTGGCGCT	5760
55	TTCTCAATGC	TCACGCTGTA	GGTATCTCAG	TTCGGTGTAG	GTCGTTCGCT	CCAAGCTGGG	5820
	CTCTCTCCAC	GAACCCCCCG	TTCAGCCCGA	CCGCTGCGCC	TTATCCGGTA	ACTATCGTCT	5880
	TCACTCCAAC	CCGGTAAGAC	ACGACTTATC	GCCACTGGCA	GCAGCCACTG	GTAACAGGAT	5940
	TACCAGACC	AGGTATGTAG	GCGGTGCTAC	AGAGTTCTTG	AAGTGGTGGC	CTAACTACGG	6000
40	CENCAGAGCG	ACCACACTAT	ТТССТАТСТС	CGCTCTGCTG	AAGCCAGTTA	CCTTCGGAAA	6060
70	A NONCOMPOCE	AGCTCTTGAT	CCGCCAAACA	AACCACCGCT	GGTAGCGGTG	GTTTTTTTGT	6120
	MMCGA ACCAC	CAGATTACGC	CCACAAAAAA	AGGATCTCAA	GAAGATCCTT	TGATCTTTTC	6180
	TIGCAAGCAG	CAGATIACGC	CCANCGAAAA	CTCACCTTAA	GGGATTTTGG	TCATGAGATT	6240
	TACGGGGTCT	DACGCICAGI	ACAMCOMMO	A A A TTO A A A A A	ATTTTSAAST	AATCAATCTA	6300
45	ATCAAAAAGG	ATCTTCACCI	AGAICCIIII	. 12012 1 1212 1 1 1 1 1 1 1 1 1 1 1 1 1	TTABTCACTC	AGGCACCTAT	6360
43	AAGTATATAT	GAGTAAACTI	GGICIGACAG	1 IACCARIGC	CERTICACIO	TGTAGATAAC	6420
	CTCAGCGATC	TGTCTATTTC	GTTCATCCAT	AGTTGCCTGA	0000000000	CACACCCACC	6480
	TACGATACGG	GAGGGCTTAC	CATCIGGCCC	CAGTGCTGCA	AIGAIACCGC	GAGACCCACG	6540
	CTCACCGGCI	CCAGATITAT	CAGCAATAAA	CCAGCCAGCC	. GGAAGGGCCG	AGCGCAGAAG	6600
	TGGTCCTGC	ACTITATCCC	CCTCCATCCA	GTCTATTAAT	TGTTGCCGGG	AAGCTAGAGT	6660
50	AAGTAGTTC	CCAGTTAATA	GTTTGCGCAA	CGTTGTTGCC	ATIGCTACAG	GCATCGTGGT	6720
	GTCACGCTCC	TCGTTTGGT	TGGCTTCATT	CAGCTCCGGT	TCCCAACGAT	CAAGGCGAGT	6780
	TACATGATCO	CCCATGTTGT	GCAAAAAAGC	GGTTAGCTCC	Treegreere	CGATCGTTGT	
	CAGAAGTAA	TTGGCCGCAC	G TGTTATCACT	CATGGTTAT	GCAGCACTGC	ATAATTCTCT	6840
_	TACTGTCATO	CCATCCGTA	GATGCTTTTC	TGTGACTGGT	GAGTACTCA	CCAAGTCATT	6900
55	CTGAGAATAC	G TGTATGCGG	GACCGAGTTO	CTCTTGCCCC	GCGTCAATA	GGGATAATAC	6960
	CGCGCCACA?	r AGCAGAACT	TAAAAGTGC	CATCATTGG	A AAACGTTCTT	CGGGGCGAAA	7020
	ACTCTCAAG	ATCTTACCG	TGTTGAGAT	CAGTTCGAT	TAACCCACTO	GTGCACCCAA	7080
	CTGATCTTC	A GCATCTTTT	CTTTCACCAC	G CGTTTCTGG	G TGAGCAAAA	A CAGGAAGGCA	7140
	AAATGCCGC	A AAAAAGGGA	TAAGGCCA	C ACGGAAATG	T TGAATACTC	A TACTCTTCCT	7200
	,22,10000						

PCT/US97/13562 WO 98/05787

```
TTTTCAATAT TATTGAAGCA TTTATCAGGG TTATTGTCTC ATGAGCGGAT ACATATTTGA
     ATGTATTTAG AAAAATAAAC AAATAGGGGT TCCGCGCACA TTTCCCCGAA AAGTGCCACC
                                                                          7320
     TGACGTCGAC GGATCGGGAG ATCTGCTAGG TGACCTGAGG CGCGCCGGCT TCGAATAGCC
     AGAGTAACCT TTTTTTTTAA TTTTATTTTA TTTTATTTTT GAGATGGAGT TTGGCGCCGA
                                                                          7440
     TCTCCCGATC CCCTATGGTC GACTCTCAGT ACAATCTGCT CTGATGCCGC ATAGTTAAGC
 5
     CAGTATCTGC TCCCTGCTTG TGTGTTGGAG GTCGCTGAGT AGTGCGCGAG CAAAATTTAA
     GCTACAACAA GGCAAGGCTT GACCGACAAT TGCATGAAGA ATCTGCTTAG GGTTAGGCGT
                                                                          7620
     TTTGCGCTGC TTCGCGATGT ACGGGCCAGA TATACGCGTT GACATTGATT ATTGACTAGT
     TATTAATAGT AATCAATTAC GGGGTCATTA GTTCATAGCC CATATATGGA GTTCCGCGTT
     ACATAACTTA CGGTAAATGG CCCGCCTGGC TGACCGCCCA ACGACCCCCG CCCATTGACG
                                                                          7800
10
     TCAATAATGA CGTATGTTCC CATAGTAACG CCAATAGGGA CTTTCCATTG ACGTCAATGG
     GTGGACTATT TACGGTAAAC TGCCCACTTG GCAGTACATC AAGTGTATCA TATGCCAAGT
                                                                          7920
     ACGCCCCCTA TTGACGTCAA TGACGGTAAA TGGCCCGCCT GGCATTATGC CCAGTACATG
                                                                          7980
     ACCITATEGE ACTITECTAC TIEGCAGTAC ATCTACETAT TAGTCATEGE TATTACCATE
     GTGATGCGGT TTTGGCAGTA CATCAATGGG CGTGGATAGC GGTTTGACTC ACGGGGATTT
                                                                          8100
15
      CCAAGTCTCC ACCCCATTGA CGTCAATGGG AGTTTGTTTT GGCACCAAAA TCAACGGGAC
                                                                          8160
      TTTCCAAAAT GTCGTAACAA CTCCGCCCCA TTGACGCAAA TGGGCGGTAG GCGTGTACGG
                                                                          8220
      TGGGAGGTCT ATATAAGCAG AGCTCTCTGG CTAACTAGAG AACCCACTGC TTACTGGCTT
                                                                          8280
      ATCGAAATTA ATACGACTCA CTATAGGGAG ACCCAAGCTT G
20
               (2) INFORMATION FOR SEQ ID NO:13:
            (i) SEQUENCE CHARACTERISTICS:
              (A) LENGTH: 8897 base pairs
              (B) TYPE: nucleic acid
25
              (C) STRANDEDNESS: single
              (D) TOPOLOGY: linear
            (ii) MOLECULE TYPE: cDNA
30
            (xi) SEQUENCE DESCRIPTION: SEQ ID NO:13:
      GACGGATCGG GAGATCTGCT AGCCCGGGTG ACCTGAGGCG CGCCGGCTTC GAATAGCCAG
      AGTAACCTTT TTTTTTAATT TTATTTTATT TTATTTTTGA GATGGAGTTT GGCGCCGATC
                                                                            120
      TCCCGATCCC CTATGGTCGA CTCTCAGTAC AATCTGCTCT GATGCCGCAT AGTTAAGCCA
35
      GTATCTGCTC CCTGCTTGTG TGTTGGAGGT CGCTGAGTAG TGCGCGAGCA AAATTTAAGC
      TACAACAAGG CAAGGCTTGA CCGACAATTG CATGAAGAAT CTGCTTAGGG TTAGGCGTTT
                                                                            300
      TGCGCTGCTT CGCGATGTAC GGGCCAGATA TACGCGTTGA CATTGATTAT TGACTAGTTA
                                                                            360
      TTAATAGTAA TCAATTACGG GGTCATTAGT TCATAGCCCA TATATGGAGT TCCGCGTTAC
                                                                            420
      ATAACTTACG GTAAATGGCC CGCCTGGCTG ACCGCCCAAC GACCCCCGCC CATTGACGTC
40
      AATAATGACG TATGTTCCCA TAGTAACGCC AATAGGGACT TTCCATTGAC GTCAATGGGT
                                                                            540
      GGACTATTTA CGGTAAACTG CCCACTTGGC AGTACATCAA GTGTATCATA TGCCAAGTAC
      GCCCCCTATT GACGTCAATG ACGGTAAATG GCCCGCCTGG CATTATGCCC AGTACATGAC
                                                                            660
      CTTATGGGAC TTTCCTACTT GGCAGTACAT CTACGTATTA GTCATCGCTA TTACCATGGT
                                                                            720
      GATGCGGTTT TGGCAGTACA TCAATGGGCG TGGATAGCGG TTTGACTCAC GGGGATTTCC
 45
      AAGTCTCCAC CCCATTGACG TCAATGGGAG TTTGTTTTGG CACCAAAATC AACGGGACTT
                                                                            840
      TCCAAAATGT CGTAACAACT CCGCCCCATT GACGCAAATG GGCGGTAGGC GTGTACGGTG
                                                                            900
      GGAGGTCTAT ATAAGCAGAG CTCTCTGGCT AACTAGAGAA CCCACTGCTT ACTGGCTTAT
       CGAAATTAAT ACGACTCACT ATAGGGAGAC CCAAGCTTGG TACCAATTTA AATTGATATC
                                                                           1020
       TCCTTAGGTC TCGAGCACCA TGAAGTTGCC TGTTAGGCTG TTGGTGCTGA TGTTCTGGAT
                                                                           1080
 50
       TCCTGCTTCC AGCAGTGATG TTGTCATGAC CCAAACCCCA CTGTCCAGTC CTGTCACGCT
                                                                           1140
       TGGACAACCT GCGTCCATCT CTTGCAGATC TAGTCAGATC ATTGTACATA ATAATGGCAA
                                                                           1200
       CACCTATCTG GAATGGTACC AGCAGAGACC AGGGCAGTCT CCACGGCTCC TGATCTACAA
                                                                           1260
       AGTITCCAAC CGATITTCIG GGGTCCCAGA CAGGITCAGC GGCAGTGGAG CTGGGACAGA
                                                                           1320
```

1380

1440

1500

1560

TTTCACACTC AAGATCAGCA GAGTGGAGGC TGAGGATGTG GGAGTTTACT ACTGCTTCCA

GGGTTCACAT GTTCCATTCA CGTTCGGCCA AGGGACAAAG TTGGAAATCA AACGTAAGTC

TCGAGTCTCT AGATAACCGG TCAATCGATT GGAATTCTAA ACTCTGAGGG GGTCGGATGA

CGTGGCCATT CTTTGCCTAA AGCATTGAGT TTACTGCAAG GTCAGAAAAG CATGCAAAGC

CCTCAGAATG GCTGCAAAGA GCTCCAACAA AACAATTTAG AACTTTATTA AGGAATAGGG

55

	GGAAGCTAGG AAGAAAC	CA AAACATCAA	G ATTITAAATI	CGCTTCTTG	G TCTCCTTGCT	1680
	AIAATTATCT GGGATAA	CA TGCTGTTT	C TGTCTGTCC	TAACATCCC	TTATION .	
	ACAACACACC CAAGGGGC	GA ACTITICITA	STABACACCE	ا خالمالمال تابال ا	~ ~~~~~~~	
_	AGGAACTGTG GCTGCACC	AT CIGICITCA	T CTTCCCGCC2	TOTATION	ACTION A S NO.	
5	TGGAACTGCC TCTGTTGT	GT GCCTGCTGA	А ТААСТТСТАТ	CCCAGAGAG	- 00333000	
	GIGGAAGGIG GATAACGC	CC TCCAATCGG	G TAACTCCCAG	GAGAGTGTC	CACACCACCA	
	GAGCAAGGAC AGCACCTA	CA GCCTCAGCA	G CACCCTGACG	CTGAGCAAAA	CACACORACO	
	GAAACACAAA GTCTACGC	CT GCGAAGTCA	C CCATCAGGGC	Cherente	COCTONACIA	2040
	GAGCTTCAAC AGGGGAGA	GT GTTAGAGGG	A GAAGTGCCCC	CIGNOCICO	CCGICACAAA	2100
10	CCTGACCCCC TCCCATCC	TT TGGCCTCTG	T CCCALAIAIAIACCCCC	ACRECIOCICE	TCAGTTCCAG	2160
	GCGGTCCTCC AGCTCATC	TT TCACCTCAC	C CCCIIIIICC	TOTAL	TACCCCTATT	2220
	AATGTTGGAG GAGAATGA	מיטאל אל מדמאל דב	2 NATOTEROGE	CCTTGGCTT	TAATTATGCT	2280
	TCATTTAATA ATTATTAT	יע הערבות היים אין היים היים היים היים היים היים היים הי	CANCELLICA	CCTGTGGTTT	CTCTCTTTCC	2340
	AATATGTAGT CATCCTAA	CC CACCTRACC	CAACTACICA	ATTTCTCTTA	TAAGGGACTA	2400
15	AATATGTAGT CATCCTAA	CACGIAACC	A TITATAAAAA	TCATCCTTCA	TTCTATTTTA	2460
•••	CCCTATCATC CTCTGCAA	SA CAGICCICC	TUAAACCCAC	AAGCCTTCTG	TCCTCACAGT	2520
	CCCCTGGGCC ATGGTAGG	AG AGACTTGCT	CCITGITITC	CCCTCCTCAG	CAAGCCCTCA	2580
	TAGTCCTTTT TAAGGGTG	AC AGGTCTTAC	A GTCATATATC	CTTTGATTCA	ATTCCCTGAG	2640
	AATCAACCAA AGCAAATT	T TCAAAAGAA	AAACCTGCTA	TAAAGAGAAT	CATTCATTGC	2700
20	AACATGATAT AAAATAAC	a cacaataaa	GCAATTAAAT	AAACAAACAA	TAGGGAAATG	2760
20	TITAAGTICA TCATGGTA	T TAGACTTAA	TATTSTAADD	CCCALD MAINTA	CR COMMON A A	2820
	CAGGTACTGA GGGACTCC	IG TCTGCCAAGO	GCCGTATTCA	GTACTTTCCA	CAACCTIAATT	2880
	TAATCCACAC TATACTGT	SA GATTAAAAA	ATTCATTALA	ATCTTCCAAA	COMMOND MAIN	2940
	AGCTGAGAGA CAAATATA	T CTATAACTC	L GCAATCCCAC	TTCTAGATYA	COVER COMMON	3000
25	CCACCCACCA AAAAACTA	NG CAAGAATGTT	CAAAGCAGCT	מאר מידידים ביד מידידים א	******	3060
25	TIGGAAATAG CCCGATTG	C CAACAATAGA	ATGAGTTATT	AAACTGTGGT	ATVOTOTA DA O	3120
	ATTAGAATAC CCAATGAG	IA GAATTAACAA	GCTACAACTA	TACCTACTCA	CACACATOAA	3180
	ICICATAAAA ATAATGTT	C ATAAGAGAAA	CTCAATGCAA	AACATATCTT	Cutation was and annual	3240
	TUATUCATAT AAAGTTCA	A ACCAGGTAAA	AATAAAGTTA	GAAATTTCCA	TYCCA A ATTENA	
	TCTTAGCTGG GGGTGGGCC	A GTTAGTGCCT	GGGAGAAGAC	AAGAAGGGGC	TTOTOGGE	3300
30	TTGGTAATGT TCTGTTCCT	C GTGTGGGGTT	GTGCAGTTAT	CATCTCTCCA	CTCTTCTCTCT	3360
	TACACATTAT GCTTCAAA	T AACTTCACAT	AAAGAACATC	TTATACCCAC	CIGITCIGIA	3420
	GAAGAGGAAT AAGTAATAG	G TCAAGACCAA	CGCAGCTGGT	AACTCCCCCC	CTCCCCATCC	3480
	ATAGCTACCT GCCTAATCO	T GCCCWCTTGA	CCCTCAATC	ANG LOGGGGC	CIGGGATCAA	3540
	AGGTGCTCAA CAAAACAAC	A GGCCTGCTAT	TTTCCTCCA	AGICIGCCTT	CCAGGGCTCA	3600
35	CTAGGAGCAC ACATACATA	G AAATTAAATC	ANNONCACACOR	TCIGIGCCCT	GTTTGGCTAG	3660
	AGAATTAACC TTGCCCAGA	C ACTGGAAACC	CATCTATCAA	CAGCAAGGG	GACAGAGGAC	3720
	GGGAAGGGCA CATGTAAAT	C VCIOOVVVCC	CAIGIAIGAA	CACTCACATG	TTTGGGAAGG	3780
	CTCTCAGCTA CTCATCCAT	C CARCACACAC	CTCATTCTAT	GGGGCACTCT	GCCCTGCCC	3840
	AAGGGGTTCA GGAGTAACT	A ACACACACCI	TTCTAAGTAC	CICICICICE	CTACACTCTG	3900
40	AAGGGGTTCA GGAGTAACT	A ACACAGCATC	CCTTCCCTCA	AATGACTGAC	AATCCCTTTG	3960
	TCCTGCTTTG TTTTTCTTT	C CAGTCAGTAC	TGGGAAAGTG	GGGAAGGACA	GTCATGGAGA	4020
	AACTACATAA GGAAGCACC	T TGCCCTTCTG	CCTCTTGAGA	ATGTTGATGA	GTATCAAATC	4080
	TTTCAAACTT TGGAGGTTT	G AGTAGGGGTG	AGACTCAGTA	ATGTCCCTTC	CAATGACATG	4140
	AACTTGCTCA CTCATCCCT	G GGGGCCAAAT	TGAACAATCA	AAGGCAGGCA	TAATCCAGTT	4200
45	ATGAATTCTT GCGGCCGCT	T GCTAGCTTCA	CGTGTTGGAT	CCAACCGCGG	AAGGGCCCTA	4260
45	TTCTATAGTG TCACCTAAA	T GCTAGAGCTC	GCTGATCAGC	CTCGACTGTG	CCTTCTAGTT	4320
	GCCAGCCATC TGTTGTTTG	C CCCTCCCCC	TCCCTTTCCTTC	ころとととでしている	CCTCCC CTC	4380
	CCACIGICCI TICCTAATA	a aatgaggaaa	TTGCATCGCA '	アアスアアアスススマア	ACCTICTON TOT	4440
	CIMITCIGGG GGGTGGGGT	G GGGCAGGACA	GCAAGGGGGA	GGATTGGGA A	GACAATAGCA	4500
50	GGCATGCTGG GGATGCGGT	G GGCTCTATGG	CTTCTGAGGC (CGAAAGAACC	AGCTYCCCCCT	4560
50	CTAGGGGGTA TCCCCACGC	G CCCTGTAGCG	GCGCATTAAG	CCCGCCCCCT	GTGGTGGTTA	
	CGCGCAGCGT GACCGCTAC	A CTTGCCAGCG	CCCTAGCGCC	CCCCCCCCCC	CCTTTCTTCC	4620
	CITCUITTUT CGCCACGTT	CGCCGGGCCTC	TCAAAAAAAGG	2224444	ATCCATORCA	4680
	ATTAGTCAGC AACCATAGT	CCCCCCTAA	CTCCCCCCAT (ACTOCATCTCA	4740
	GTTCCGCCCA TTCTCCGCC	CATGGCTGAC	TARTHUM (TATTOLCCCIA	ACTUCUCUCA	4800
55	CCGCCTCGGC CTCTGAGCT	TTCCACAACT	PCACTATITIES	ADDINITION	GAGGCCGAGG	4860
	TTTGCAAAAA GCTTGGACA	CLCFCCC.	CC A TOTO CONCOC (COLLITIUGA	GGCCTAGGCT	4920
	AGCGTGAAGG CTGGTAGGA		CONTITION (CAAACTIGA	CGCCAATCCT	4980
	TCGTCGCCGT GTCCCAAAA	T ATCCCCCG	CONNECTION (GITCGACCA	TIGAACTGCA	5040
	TCGTCGCCGT GTCCCAAAA	TTCCESSOR	GCAAGAACGG 1	AGACCTACCC	TGGCCTCCGC	5100
	TCAGGAACGA GTTCAAGTA	LICCAAAGAA	TGACCACAAC (TCTTCAGTG	GAAGGTAAAC	5160

	TOTAL TOTAL ANTICALCAT	5220
	AGAATCTGGT GATTATGGGT AGGAAAACCT GGTTCTCCAT TCCTGAGAAG AATCGACCTT	5280
	THE PARTY OF THE PROPERTY OF THE PARTY OF TH	5340
	TARGETTAL IGENERACE STREET	5400
		5460
5		5520
•		5580
		5640
		_
		5700
10	conscions Character Addictand Cinesian	5760
10		5820
		5880
		5940
		6000
1.5	AND CARCELLINE TO THE COLUMN CONTRACTOR	6060
15	AND	6120
		6180
		6240
	TACABLE TO THE PROPERTY OF THE	6300
••	ANNINGTON CACACCTON LLIGANCEIG AMAGENTA	6360
20	TAGAGGTTTT ACTTGCTTTA AAAAACCTCC CCACACTTA TAATGGTTAC AAATAAAGCA TGAATGCAAT TGTTGTTGTT AACTTGTTTA TTGCAGCTTA TAATGGTTAC AAATAAAGCA	6420
	TGAATGCAAT TGTTGTTGTT AACTIOTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTCACA AAATTTCACA AATAAAGCAT TTTTTTCACT GCATTCTTG CAGCGGGGG	6480
	ATAGCATCAC AAATTTCACA AATAAASCAT GGATCGGCTG GATGATCCTC CAGCGCGGGG CCAAACTCAT CAATGTATCT TATCATGTCT GGATCGGCTG GATGATCCTC CAGCGCGGGG	6540
	CCAAACTCAT CAATGTATCT TATCATGTAT TGCAGCTTAT AATGGTTACA ATCTCATGCT GGAGTTCTTC GCCCACCCCA ACTTGTTTAT TGCAGCTTAT AATGGTTACA	6600
	ATCTCATGCT GGAGTTCTTC GCCCACCCCA ACTICATOR TTTTTCACTG CATTCTAGTT AATAAAGCAA TAGCATCACA AATTTCACAA ATAAAGCATT TTTTTCACTG CATTCTAGCT	6660
25	AATAAAGCAA TAGCATCACA AATTICACAT ATAACCGTCG ACCTCTAGCT GTGGTTTGTC CAAACTCATC AATGTATCTT ATCATGTCTG TATACCGTCG ACCTCTAGCT	6720
	GTGGTTTGTC CAAACTCATC AATGTATCTT ATCATGTGTG AAATTGTTAT CCGCTCACAA AGAGCTTGGC GTAATCATGG TCATAGCTGT TTCCTGTGTG AAATTGTTAT CCGCTCACAA	6780
	AGAGCTTGGC GTAATCATGG TCATAGCTGT TTCCTGTGTG ANGCCCTGGGGTGCC TAATGAGTGA	6840
	AGAGCTTGGC GTAATCAIGG ICAAAGCATAA AGTGTAAAGC CTGGGGTGCC TAATGAGTGA TTCCACACAA CATACGAGCC GGAAGCATAA AGTGTAAAGC CTGGGGTGCC TAATGAGTGA	6900
	TTCCACACAA CATACGAGCC GGAAGCATAC TGCCCGCTTT CCAGTCGGGA AACCTGTCGT GCTAACTCAC ATTAATTGCG TTGCGCTCAC TGCCCGCTTT CCAGTCGGGA AACCTGTCGT GCTAACTCAC ATTAGTGCG TTGCGCTCAC TGCCCGCTTTTGCGT ATTGGGCGCT	6960
30	GCTAACTCAC ATTAATTGCG TIGGGCCAACGCG CGGGGAGAGG CGGTTTGCGT ATTGGGCGCT GCCAGCTGCA TTAATGAATC GGCCAACGCG CGGGGAGAGG CGGTTTGCGG CGAGCGGTAT	7020
	GCCAGCTGCA TTAATGAATC GGCCAGCTGC GCTCGGTCGT TCGGCTGCGG CGAGCGGTAT CTTCCGCTTC CTCGCTCACT GACTCGCTGC GCTCGGTCGT TCGGCTGCGG CGAGCAAGA	7080
	CTTCCGCTTC CTCGCTCACT GACCGGTTAT CCACAGAATC AGGGGATAAC GCAGGAAAGA CAGCTCACTC AAAGGCGGTA ATACGGTTAT CCACAGAATC AGGGGATAAC GCAGGAAAGA CAGCTCACTC AAAGGCCGGTA ATACGGTTAT AAAGGCCGCG TTGCTGGCGT	7140
	CAGCTCACTC AAAGGCCGTA ATACGCCA GGAACCGTAA AAAGGCCGCG TTGCTGGCGT ACATGTGAGC AAAAGGCCAG CAAAAGGCCA GGAACCGTAA AAAGGCCGCG TTGCTGGCGT	7200
	ACATGTGAGC AAAAGGCCAG CAAAAGGCCA ATCACAAAAA TCGACGCTCA AGTCAGAGGT TTTTCCATAG GCTCCGCCCC CCTGACGAGC ATCACAAAAA TCGACGCTCA AGTCAGAGGT	7260
35	TTTTCCATAG GCTCCGCCCC CCIGACGACCC AGGCGTTTCC CCCTGGAAGC TCCCTCGTGC GGCGAAACCC GACAGGACTA TAAAGATACC AGGCGTTTCC CCCTTCTCGGAA	7320
	GGCGAAACCC GACAGGACIA TAMAGATACC GATACCTGTC CGCCTTTCTC CCTTCGGGAA GCTCTCCTGT TCCGACCCTG CCGCTTACCG GATACCTGTC CGCCTTTCTC CTTCGGGAA	7380
	TO TOTAL TOTAL TOTAL CONTENT CONTENTS OF THE PROPERTY OF THE P	7440
	GCGTGGCGCT TTCTCAATGC TCACGCTCCG TTCAGCCCGA CCGCTGCGCC TTATCCGGTA CCAAGCTGGG CTGTGTGCAC GAACCCCCCG TTCAGCCCGA CCGCTGCGCA CCGCCACTG	7500
	TO THE PROPERTY OF THE PROPERT	7560
40	ACCTATCTAG GUIGGIAL AGAGIICITO ALGORIO	7620
	THE THE PROPERTY AND A RECEIVED AND CONTRACT OF THE COURSE	
	CTAACTACGG CTACACTAGA AGGACCAGTAT CCTTCGGAAA AAGAGTTGGT AGCTCTTGAT CCGGCAAACA AACCACCGCT GGTAGCGGTG CCTTCGGAAA AAGAGTTGGT AGCTCTTGAT CCGGCAAACA AACCACCGCT GGTAGCGGTG	7740
		7800
	TARGERET CACCETTAGT GGARGIAAAA CICACGIIAA OOMIIII	7860
45		7920
	TACCAMIC TIMES	7980
		8040
	THE PROPERTY OF THE PARTY OF TH	8100
	THE PROPERTY OF THE PARTY OF TH	8160
50	TOTAL TO THE PROPERTY OF A CONTRACT OF COTOCATOCA GIUNIANI IGIIGCOGG	8220
	CARTTANTA GTTTGCGCAA CGIIGIIGCC AIICEINGIG	8280
	TOTAL TOTAL COMMON TOCOTTOCATA TOCOTTOCATA CAGCILLOGI ICCURSIONI	8340
		8400
	THE PART OF THE PROPERTY OF THE PARTY OF THE	8460
55	The second of the control of the con	
55	TOTAL CONTROL OF THE PROPERTY	8520
	TARABETTE CALCACION TO ACCREDIT TARABETTE CALCALIGOR PERSON	8580
	TOTAL STATE OF THE PROPERTY OF	8640
	CGGGGCGAAA ACTCICAGG ATCTTACCO CTTTCACCAG CGTTTCTGGG TGAGCAAAAA	8700
	w	

CAGGAAGGCA A	LAATGCCGCA	AAAAAGGGAA	TAAGGGCGAC	ACGGAAATGT	TGAATACTCA	8760
TACTCTTCCT 1	TTTCAATAT	TATTGAAGCA	TTTATCAGGG	TTATTGTCTC	ATGAGCGGAT	8820
ACATATTTGA A	TGTATTTAG	AAAAATAAAC	AAATAGGGGT	TCCGCGCACA	TTTCCCCGAA	8880
AAGTGCCACC T	GACGTC					8897

What is claimed is:

- 1. A method for inhibiting immunoglobulin-induced toxicity resulting from

 immunoglobulin immunotherapy in a subject comprising administering an

 immunoglobulin molecule to the subject, the immunoglobulin molecule

 having a variable region and a constant region, the immunoglobulin molecule

 being modified prior to administration by structurally altering multiple

 toxicity associated domains in the constant region so that immunoglobulin
 induced toxicity is inhibited.
- A method for inhibiting immunoglobulin-induced toxicity resulting from immunoglobulin immunotherapy in a subject comprising administering a structurally altered antibody to the subject, the structurally altered antibody comprising a variable region and a constant region, multiple toxicity associated domains in the constant region being modified so as to render the constant region unable to mediate an ADCC response or activate complement thereby inhibiting immunoglobulin-induced toxicity resulting from immunotherapy.

20

3. A method for inhibiting immunoglobulin-induced toxicity resulting from immunotherapy in a subject comprising administering an Ig fusion protein to the subject, the Ig fusion protein having multiple structurally altered toxicity associated domains in the constant region.

25

4. A method for inhibiting immunoglobulin-induced toxicity resulting from immunotherapy in a subject comprising administering an Ig fusion protein to the subject, the Ig fusion protein comprising a modified constant region, the

modification being a structural alteration in multiple toxicity associated regions within the CH₂ domain.

- A method for preventing immunoglobulin-induced toxicity resulting from
 immunotherapy for a disease in a subject comprising:
 - selecting an immunoglobulin which recognizes and binds a target, the target being associated with the disease;
- 10 (b) mutating the immunoglobulin so selected by structurally altering multiple toxicity associated domains in the constant region of the immunoglobulin thereby creating a structurally altered immunoglobulin;
- 15 (c) administering the structurally altered immunoglobulin of step (b) to the subject under conditions so that the structurally altered immunoglobulin recognizes and binds the target thereby alleviating symptoms associated with the disease, the structural alteration of the constant region thereby preventing immunoglobulin-induced toxicity in the subject.
 - 6. A method for preventing immunoglobulin-induced toxicity resulting from immunotherapy for a disease in a subject comprising:
- 25 (a) selecting an Ig fusion protein which recognizes and binds a target, the target being associated with the disease;
 - (b) structurally altering multiple toxicity associated domains in the CH₂ domain of the constant region of the Ig protein so selected;

5

15

- (c) administering the structurally altered Ig fusion protein of step (b) to the subject under conditions so that the structurally altered Ig fusion protein recognizes and binds the target thereby alleviating symptoms associated with the disease, the structural alteration of the CH₂ domain thereby preventing immunoglobulin-induced toxicity in the subject.
- 7. The method of claim 1, 2, 3, 4, 5, or 6, wherein the portion of the constant region is the CH₂ domain.
 - 8. The method of claim 1 or 5, wherein the immunoglobulin molecule is IgG.
 - 9. The method of claim 1 or 5, wherein the immunoglobulin molecule is IgM.
 - 10. The method of claim 1 or 5, wherein the immunoglobulin molecule is IgA.
 - 11. The method of claim 2, wherein the antibody recognizes and binds Le^y.
- 20 12. The method of claim 2, wherein the antibody recognizes and binds to Lex.
 - 13. The method of claim 2, wherein the antibody is a monoclonal antibody BR96 produced by the hybridoma having the identifying characteristics of HB 10036 as deposited with the ATCC.
 - 14. The method of claim 2, wherein the antibody is a chimeric antibody

 ChiBR96 produced by the hybridoma having the identifying characteristics

 of HB 10460 as deposited with the ATCC.

15. The method of claim 1 or 5, wherein the immunoglobulin recognizes and binds Le^y.

- The method of claim 1 or 5, wherein the immunoglobulin recognizes and
 binds to Le^x.
 - 17. The method of claim 1 or 5, wherein the immunoglobulin is a monoclonal antibody BR96 produced by the hybridoma having the identifying characteristics of HB 10036 as deposited with the ATCC.

10

- 18. The method of claim 1 or 5, wherein the immunoglobulin is a chimeric antibody ChiBR96 produced by the hybridoma having the identifying characteristics of HB 10460 as deposited with the ATCC.
- 15 19. The method of claim 3, 4, or 6, wherein the Ig fusion protein recognizes and binds Le^y.
 - 20. The method of claim 3, 4, or 6, wherein the Ig fusion protein recognizes and binds to Le^x.

- 21. The method of claim 3, 4, or 6, wherein the Ig fusion protein is a derivative of monoclonal antibody BR96 produced by the hybridoma having the identifying characteristics of HB 10036 as deposited with the ATCC.
- 25 22. The method of claim 3, 4, or 6, wherein the Ig fusion protein is a derivative of chimeric antibody ChiBR96 produced by the hybridoma having the identifying characteristics of HB 10460 as deposited with the ATCC.
 - 23. A pharmaceutical composition comprising a pharmaceutically effective

amount of a structurally altered immunoglobulin, and an acceptable carrier, the structurally altered immunoglobulin (1) recognizes and binds a target, the target is associated with cancer and (2) has an inactivated CH₂ domain.

- A pharmaceutical composition comprising a pharmaceutically effective amount of structurally altered Ig fusion protein, and an acceptable carrier, the structurally altered Ig fusion protein (1) recognizes and binds a target, the target is associated with cancer and (2) has an inactivated CH₂ domain.
- 10 25. A method of treating carcinomas in vivo comprising administering to a subject a pharmaceutically effective amount of the composition of claim 23 or 24.
- The method of claim 30, wherein the structurally altered immunoglobulin in the composition is labeled so as to directly or indirectly produce a detectable signal with a compound selected from the group consisting of a radiolabel, an enzyme, a chromophore, a chemiluminescer, and a fluorescer.
- The method of claim 24, wherein the Ig fusion protein in the composition is labeled so as to directly or indirectly produce a detectable signal with a compound selected from the group consisting of a radiolabel, an enzyme, a chromophore, a chemiluminescer, and a fluorescer.
- 28. The method of claim 2 or 5, wherein the antibody is conjugated to a cytotoxic agent.
 - 29. The method of claim 1, wherein the immunoglobulin is conjugated to a cytotoxic agent.

30. The method of claim 3, 4 or 6, wherein the lg fusion protein is conjugated to a cytotoxic agent.

- The method of claim 28, 29, or 31, wherein the cytotoxic agent is selected
 from the group consisting of antimetabolites, alkylating agents,
 anthracyclines, antibiotics, anti-mitotic agents, and chemotherapeutic agents.
- 32. A method for treating a subject suffering from a cancer, the cancer being characterized as a group of cells having a tumor associated antigen on the cell surface, which method comprises administering to the subject a cancer killing amount of the composition of claim 23 or 24 joined to a cytotoxic agent under conditions which permit the molecule so joined to bind the tumor associated antigen on the cell surface so as to kill the cells so bound thereby curing the subject.
 - 33. A pharmaceutical composition comprising a pharmaceutically effective amount of a structurally altered BR96 antibody, the structurally altered antibody having an inactivated CH₂ domain.

20

- 34. A method for treating a subject suffering from a proliferative type disease characterized by cells having a BR96 antigen on the cell surface which comprises administering to the subject an effective amount of the composition of claim 33 joined to doxorubicin such that the immunoconjugate binds the BR96 antigen and kills said cells thereby treating the subject.
- 35. A method for inhibiting BR96 (ATCC: HB10036) induced toxicity resulting from immunoglobulin immunotherapy in a subject comprising administering

BR96 to the subject, the BR96 molecule being modified prior to administration, the modification comprising the deletion or substitution of at least one amino acid residue in the toxicity associated domain localized to amino acids 310-331 and the deletion or substitution of at least one amino acid residue in the toxicity associated domain localized to amino acids 231-238 so that complement and Fc receptor mediated toxicity is inhibited.

36. A method for preventing BR96 (ATCC: HB10036) induced toxicity resulting from immunotherapy for cancer in a subject comprising:

5

10

- (a) mutating the BR96 polypeptide by the deletion or substitution of at least one amino acid residue in the toxicity associated domain localized to amino acids 310-331 and the deletion or substitution of at least one amino acid residue in the toxicity associated domain localized to amino acids 231-238 so that complement and Fc receptor mediated immunoglobulin-induced toxicity is inhibited in the altered BR96 polypeptide; and
- (b) administering the structurally altered BR96 polypeptide of step (a) to
 the subject under conditions so that the peptide recognizes and binds
 cancer associated Le^y antigens, thereby alleviating symptoms
 associated with the cancer, the structural alteration of the toxicity
 associated domains thereby preventing BR96 toxicity in the subject.
- 25 37. A chimeric BR96 antibody having a structurally altered constant region having the CH1 and CH3 domains but not the CH2 domain, the antibody being designated cBR96-A.

38. The chimeric BR96 antibody of claim 37 which is expressed by the plasmid having the sequence shown in SEQ ID NO. 10.

- 39. A BR96 antibody having humanized variable and constant regions, wherein the constant region has been structurally altered so that the CH1 and CH3 domains are present but the CH2 domain is not, the antibody being designated hBR96-2A.
- 40. The BR96 antibody of claim 39 which is expressed by the plasmid having the sequence shown in SEQ ID NO. 12.
 - 41. A BR96 antibody designated hBR96-2B having a structurally altered constant region wherein leucine at amino acid position 235 is mutated to alanine and glycine at amino acid position 237 is mutated to alanine.
- 42. A BR96 antibody designated hBR96-2C having a structurally altered constant region wherein glutamic acid at amino acid position 318 is mutated to serine; lysine at amino acid position 320 is mutated to serine; and lysine at amino acid position 322 is mutated to serine.
 - 43. A BR96 antibody designated hBR96-2D having a structurally altered constant region wherein proline at amino acid position 331 is mutated to alanine.

20

25 44. A BR96 antibody designated hBR96-2E having a structurally altered constant region wherein leucine at amino acid position 235 is mutated to alanine; glycine at amino acid position 237 is mutated to alanine; glutamic acid at amino acid position 318 is mutated to serine; lysine at amino acid

position 320 is mutated to serine; and lysine at amino acid position 322 is mutated to serine.

- 45. A BR96 antibody designated hBR96-2F having a structurally altered
 5 constant region wherein leucine at amino acid position 235 is mutated to
 alanine; glycine at amino acid position 237 is mutated to alanine; and proline
 at amino acid position 331 is mutated to alanine.
- 46. A BR96 antibody designated hBR96-2G having a structurally altered

 constant region wherein glutamic acid at amino acid position 318 is mutated
 to serine; lysine at amino acid position 320 is mutated to serine; and lysine at
 amino acid position 322 is mutated to serine; and proline at amino acid
 position 331 is mutated to alanine.
- A BR96 antibody designated hBR96-2H having a structurally altered constant region wherein leucine at amino acid position 235 is mutated to alanine; glycine at amino acid position 237 is mutated to alanine; glutamic acid at amino acid position 318 is mutated to serine; lysine at amino acid position 320 is mutated to serine; lysine at amino acid position 322 is mutated to serine; and proline at amino acid position 331 is mutated to alanine.
 - 48. A nucleic acid molecule which encodes the BR96 antibody of claim 37, 39, and 41-47.
 - 49. A cDNA of claim 48.

25

50. A plasmid which comprises the nucleic acid molecule of claim 48.

51. A host vector system comprising a plasmid of claim 50 in a suitable host cell.

52. A method for producing a protein comprising growing the host vector system of claim 51 so as to produce the protein in the host and recovering the protein so produced.

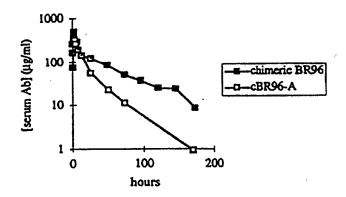


Figure 1. Plasma clearance in high LeY expressing dogs chimeric versus constant region mutant of cBR96-2.

Figure One

Figure 2

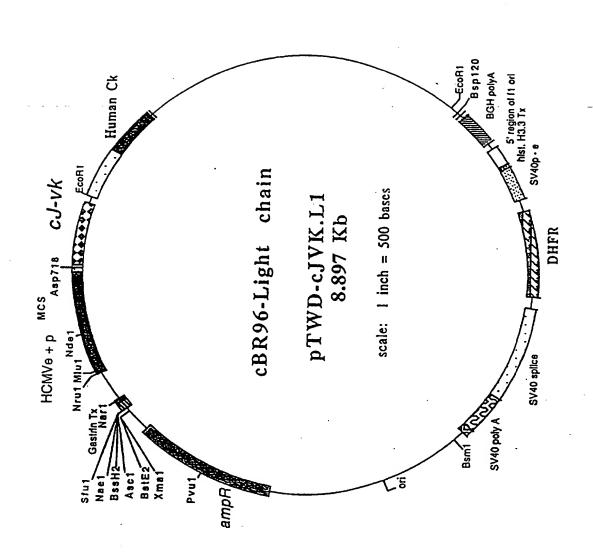


Figure 3

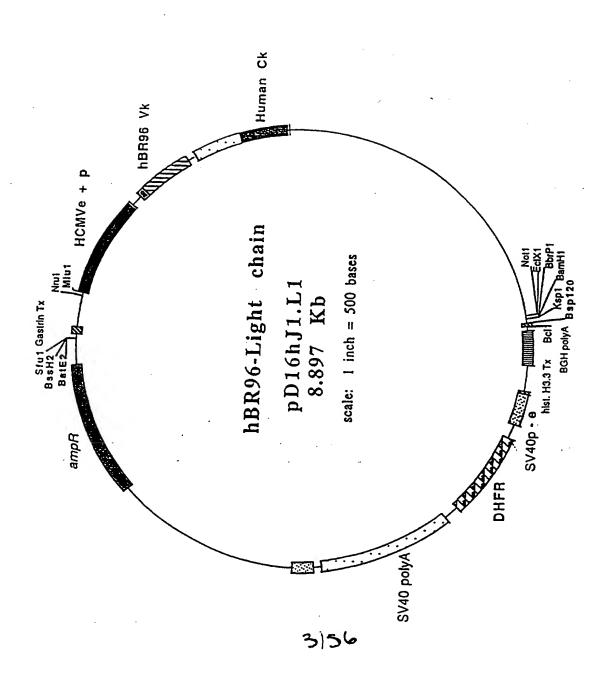


Figure 4

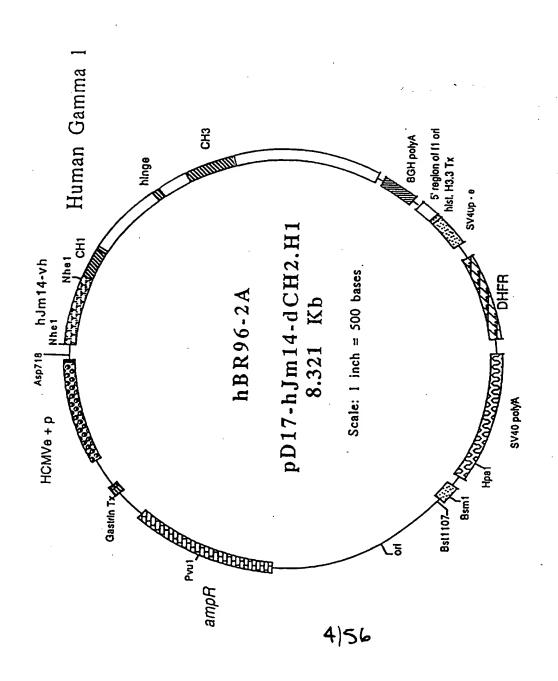


Figure 5

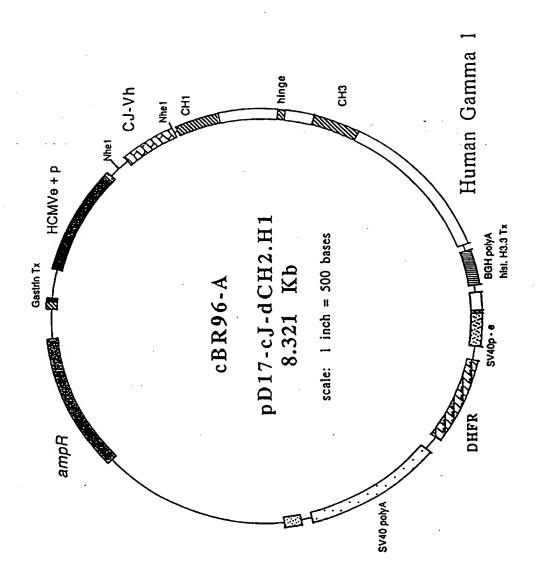


Figure 6

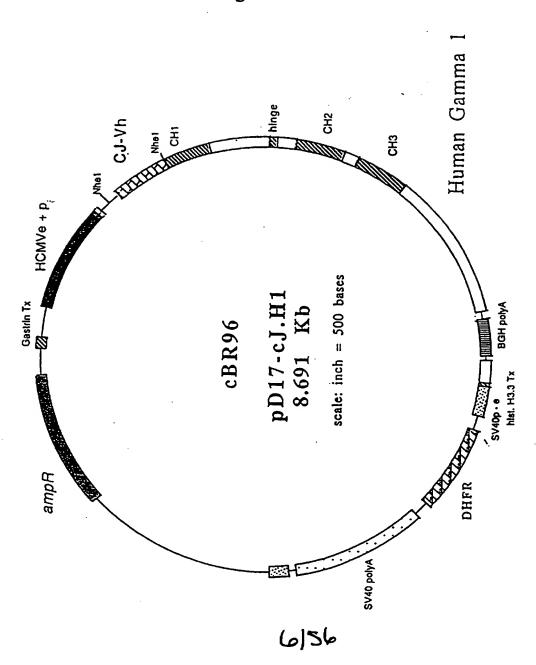


Figure 7

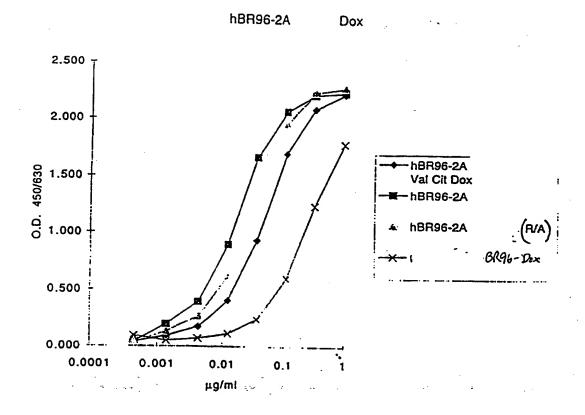
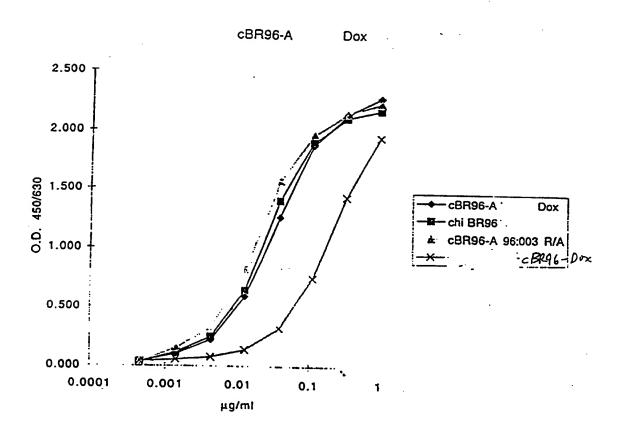
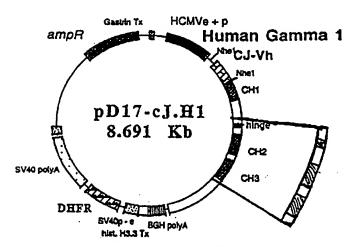


Figure 8



A- Hinge + Cl... + CH3 domains were removed from R96 IgG1 construct by E.co -III restriction digestion.



8.2. Hinge + CH3 domains amplified by PCR from L6 IgG1 construct lacking the CH2 domain.

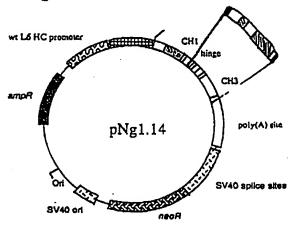


Figure 9

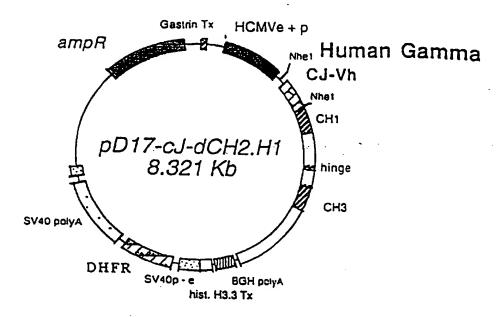
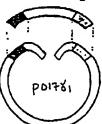


Figure 9 (CONTINUED)

- 1- Introduction of mutations by site-directed mutagenesis on double-stranded plasmid DNA.
- A- Mutations introduced into synthetic oligonucleotides used for the PCR amplification of CH2 domain.

B- Plasmid DNA linearized inside CH2 domain and cotransformed with PCR fragment into competent DH5 α .

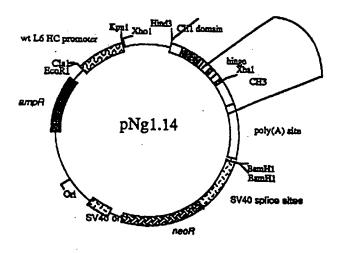


C- Cloning mediated by homologous recombination yields transformants harbouring recombinant plasmids.



Figure 10

Figure 11



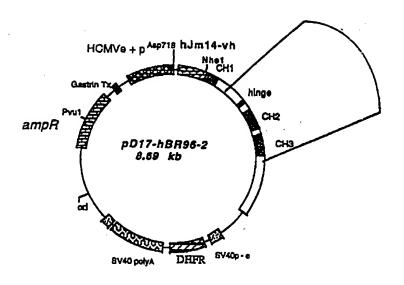


Figure 12

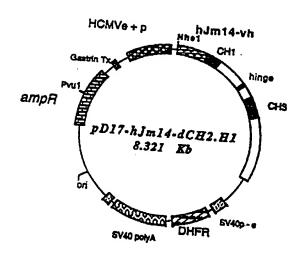


Figure 13

					•				
90	180	270	360	450	540	630	720	810	820 830 830 840 850 850 860 870 870 880 890 900 TTTGGCACC CATTGGCACC TAGGCGTGTA CGGTGGGAGG AAATGGGCG TAGGCGTGTA CGGTGGGAGG AAATGGCGCG TAGGCGTGTA CGGTGGGAGG AAATGGCGCG TATAGTTGCC CTGAAAGGTT TTACAGCATT GTTGAGGCGG GGTAACTGCG TTTACCCGCC ATCGGCACAT GCCACCTCC
TTATTTTATT	AGCCAGTATC	AATTGCATGA	AGTTATTAAT	GGCTGACCGC	TGGGTGGACT	AAATGGCCCG	ATGGTGATGC	GGGAGTITGT	
AATAAAATAA	TCGGTCATAG	TTAACGTACT	TCAATAATTA	CCGACTGGCG	ACCCACCTGA	TYTACCGGGC	TACCACTACG	CCCTCAAACA	
80	160	260	350	440	530	620	710	800	890
Taattttatt	GCTCTGATGC CGCATAGTTA AGCCAGTATC	CTTGACCGAC	ATTATTGACT	TGGCCCGCCT	TTGACGTCAA	CAATGACGGT	CGCTATTACC	TGACGTCAAT	TAGGCGTGTA
Attaaaataa	CGAGACTACG GCGTATCAAT TCGGTCATAG	GAACTGGCTG	TAATAACTGA	ACCGGGCGGA	AACTGCAGTT	GTTACTGCCA	GCGATAATGG	ACTGCAGTTA	ATCCGCACAT
70 CCITIVITITI GGAAAAAAA	160 GCTCTGATGC CGAGACTACG	250 CAAGGCAAGG GTTCCGTTCC	340 GTTGACATTG CAACTGTAAC	TTACGGTAAA AATGCCATTT	520 GGACTTTCCA CCTGAAAGGT	610 CTATTGACGT GATAACTGCA	700 TATTAGTCAT ATAATCAGTA	790 TCCACCCCAT AGGTGGGGTK	880 AAATGGGCGG TTTACCCGCC
60	150	240	330	420	510	600	690	780	870
GCCAGAGTAA	AGTACAATCT	TAAGCTACAA	AGATATACGC	GETACATAAC	ACGCCAATAG	AGTACGCCCC	TACATCTACG	TTTCCAAGTC	CCATTGACGC
CGGTCTCATT	TCATGTTAGA	ATTCGATGTT	TCTATATGCG	CAATGTATTG	TGCGGTTATC	TCATGCGGGG	ATGTAGATGC	AAAGGTTCAG	GGTAACTGCG
50	140	230	320	410	500	590	680	770	860
GCTTCGAATA	GTCGACTCTC	GAGCAAAATT	TGTACGGGCC	GGAGTTCCGC	TCCCATAGTA	TCATATGCCA	TACTTGGCAG	CTCACGGGGA	CAACTCCGCC
CGAAGCTTAT	CAGCTGAGAG	CTCGTTTTAA	ACATGCCCGG	CCTCAAGGCG	AGGGTATCAT	AGTATACGGT	ATGAACCGTC	GAGTGCCCCT	GTTGAGGCGG
10 20 30 30 40 50 60 60 70 70 80 90 90 GACGGATCGG GAGATCTGCT AGGTGACCTG AGGCGCGCG GCTTCGAATA GCCAGAGTAA CCTTTTTTTT TAATTTTATT TTATTTTATT	110 150 150 150 170 170 180 180 180 180 180 180 180 180 180 18	190 240 250 250 250 250 250 250 250 250 250 25	280 290 350 350 350 350 ACTIVECO TOCITICECOR TOPACOGOCC AGAININACE CITCACATIG ALTAITIGACT AGITATIAAT ACAANCIC GOAAAACGCG ACGAAGCCCG TOTACCGG TOTALATICCG CAACTGIAAC TAATAACGCG ACGAAGCGCT ACAIGACGG TOTALATICCG CAACTGIAAC TAATAACGCG ACGAAGCGCT ACAIGCCG TOTALATAGCG CAACTGIAAC TAATAACTGA TOAATAATTA	370 480 440 440 440 450 ACCCATATAT GGAGTTCCGC GTTACATAAC TTACGGTAAA TGGCCCGCCT GGCTGACCGC GTTACATATA TACGCCAGGCT GGCTGACGGC GTTACATATA ATGCCCCAGT AATCAAGTAT CGGGTATATA CCTCAAGGC CAATGTATTG AATGCCCAGT AATCAAGTAT CGGGTATATA CCTCAAGGC CAATGTATTG AATGCCCAAT ACGGGGGGG CGACTGGCG	460 470 480 580 590 590 590 590 590 590 540 540 540 590 590 590 590 590 590 590 590 590 59	550 550 560 570 570 580 570 580 570 580 600 600 610 620 620 620 ATTACGGTA AACTGCCCC CTATTGACGT CAATGACGGT AAATGGCCCG TAAATGACGGT GAATGACGGT AAATGGCCGG TAAATGCCCG TAAATGCCGG GATAACTGCA AACCGTCATG TAGTTCACAT AGTATACGGT TCATGCGGGG GATAACTGCA GTTACTGCCA TTTACCGGGG	650 660 710 710 720 CCTGGGATTTC TACTTGGCAG TACATCTACG TATTAGTCAT CGCTATTACC ATGGTGATGG CGGGTCATA ACGGGATCAT CCCTGAAAGG ATGAACGTC ATGTGATGG ATGTAGATGG ATGTAGATGG ATGTAGATGG ATGTAGATGG ATGTAGATGA ACGGGTCATG TACTGGAATA CCCTGAAAGG ATGAACCGTC ATGTAGATGG ATGAACGATGATG TACTGGAATA CCCTGAAAAGG ATGAACCGTC ATGTAGAATGG ATGAACGATGAATGG TACCACTACG	710 780 790 800 800 810 GGTTTTG 770 780 790 790 800 800 810 GGTTTTGT GGCGTGGAT AGGGGTTTTGA CTCACGCGA TTTCCAAGTC TCCACCCCAT TGACGTCAAT GGGGGTTTTGT CCAAAACCG CATGTAGTTA CCGCACCTA TCGCCAAACT GAGTGCCCT AAAGGTTCAG AGGTGGGGTA ACTGCAGTTA CCCTCAAACA	840 850 860 870 880 GACTITICCAA AAIGICGIAA CAACICCGCC CCATIGACGC AAAIGGGCGG CIGAAAGGIT TIACAGCAIT GIIGAGGCGG GGIAACIGCG ITTACCCGCC
30	120	210	300	390	480	570	660	750	840
AGGTGACCTG	CGATCTCCCG	GAGGTCGCTG	CGTTTTGCGC	TTAGTTCATA	ACGTCAATAA	TTGGCAGTAC	ATGACCTTAT	GGGCGTGGAT	GACTITICGAA
TCCACTGGAC	GCTAGAGGGC	CTCCAGCGAC	GCAAAACGCG	AATCAAGTAT	TGCAGTTATT	AACCGTCATG	TACTGGAATA	CCCGCACCTA	CTGAAAGGIT
20 GAGATCTGCT CTCTAGACGA	110 AGTTTGGCGC TCAAACCGCG	200 TTGTGTGTTG AACACACAAC	290 TAGGGTTAGG ATCCCAATCC	380 TACGGGGTCA ATGCCCCAGT	470 CCGCCCATTG GGCGGGTAAC	560 AACTGCCCAC TTGACGGTG	650 TGCCCAGTAC ACGGTCATG	740 GTACATCAAT CATGTAGTTA	820 TTTGGCACCA NATCAACGG
10 GACGGATCGG CTGCCTAGCC	100 TTTGAGATGG AACTCTACC	190 TGCTCCCTGC ACGAGGGACG	280 AGAATCTGCT TCTTAGAGGA	370 AGTAATCAAT TCATTAGTTA	460 CCAACGACCC GGTTGCTGGG	S50 ATTTACGGTA TAAATGCCAT	640 CCTGGCATTA GGACCGTAAT	730 GGTTTTGGCA CCAAAACCGT	820 TTTGGCACCA

990 GAGACCCAAG CTCTGGGTTC 1080 GCTTGCTAGC	CGAACGATCG 1170 TCTGGGGGAG AGACCCCTC	1260 GTTCGCCAGA CAAGCGGTCT	1350 CGATTCACCA GCTAAGTGGT	1440 GCAAGAGGCC CGTTCTCCGG	1530 GTCTTCCCCC CAGAAGGGGG	1620 ACGGTGTCGT TGCCACAGCA	1710 GTGGTCACCG CACCAGTGGC	1800 GTTGGTGAGA CAACCACTCT
970 980 TTAATACGAC TCACTATAGG AATTATGCTG AGTGATATCC 1060 1070 CGATTGGAAT TCTTGCGGCC	AGAACGCCGG 1160 TCTGGTGGAG AGACCACCTC	1250 CATGTATTGG GTACATAACC	1340 TCTAAAGGGT ACATTTCCCA	1430 GTATTACTGT CATAATGACA	1520 GGGCCCATCG CCCGGGTAGC	1610 CGAACCGGTG GCTTTGGCCAC	1700 CCTCAGCAGC GGAGTCGTCG	1790 GGACAAGAAA CCTGTTCTTT
970 TTAATACGAC AATTATGCTG 1060 CGATTGGAAT	GCTAACCTTA 1150 GTGAAGTGAA CACTTCACTT	1240 GTGACTATTA CACTGATAAT	1330 ATCCAGACAC TAGGTCTGTG	1420 ACACAGCCAT TGTGTCGGTA	1510 CTAGCACCAA GATCGTGGTT	1600 ACTACTTCCC TGATGAAGGG	1690 1700 GACTCTAÇTC CCTCAGCAGC CTGAGATGAG GGAGTCGTCG	ACACCAAGGT GGACAAGAAA TGTGGTTCCA CCTGTTCTTT
950 960 960 ACCETACTOG CTTATCGAAA ACGAATGACC GAATAGCTTT 1040 1050 TCTCTAGATA ACCGGTCAAT	TGGCCAGTTA 1140 GGTGTCCAGT CCACAGGTCA	1230 TTCACTTTCA AAGTGAAAGT	1320 ATAACCGACT TATTGGCTGA	1410 AAGTCTGAGG TTCAGACTCC	1500 GTCTCTGTAG CAGAGACATC	1590 1600 CGGCCAAGG ACTACTTCCC CGAACCGGTG GACCAGTTCC TGATGAAGGG GCTTTGGCCAC	1680 CAGTCCTCAG GTCAGGAGTC	AAGCCCAGCA TTCGGGTCGT
950 TGCTTACTGG ACGAATGACC 1040 TCTCTAGATA	AGAGATCTAT 1130 TGTTTTAAAA ACAAAATTTT	1220 AACCTCTGGA TTGGAGACCT	1310 AGGTGGTGAT TCCACCACTA	1400 GAGCCGTCTG CTCGGCAGAC	1490 TCTGGTCACG AGACCAGTGC	1580 CCTGGGCTGC GGACCCGACG	1670 GGCTGTCCTA CCGACAGGAT	1760 CGTGAATCAC GCACTTAGTG
940 GAGAACCCAC CTCTTGGGTG 1030 AGGTCTCGAG	TAAATTTAAC TATAGAGGAA TCCAGAGCTC AGAGATCTAT TGGCCAGTTA GCTAACCTTA AGAAGGCGG CGAAGGATCG 1100 1110 1110 1120 1120 1130 1140 TTGTGGTTAA GCTTGGTCT TCCTTGTCCT TGTTTTAAA GGTGTCCAGT GTGAAGTGAA	1200 1250 1250 1250 1250 1250 1260 1250 1250 1260 1250 1260 1260 1260 1260 1260 1260 1260 126	1300 ACATTAGTCA TGTAATCAGT	1390 ACCTGCAAAT TGGACGTTTA	1470 1500 1520 1520 CCAAGGGAC TCTGGTCACG GTCTCTGTAG CTAGCACCAA GGGCCCATCG CGAATGACCC CGGTTCCCTG AGACCAGTGC CAGAGACATC GATCGTGGTT CCCGGGTAGC	1560 1570 1580 ACCTCTGGGG GCACAGCGGC CCTGGGCTGC TGGAGACCCC CGTGTCGCCG GGACCCGACG	ACACCTTCCC GGCTGTCCTA CAGTCCTCAG TGTCGAAGGG CCGACAGGAT GTCAGGAGTC	ACCCAGACCT ACATCTGCAA CGTGAATCAC AAGCCCCAGGA TGGGTCTGGA TGTAGACGTT GCACTTAGTG TTCGGGTCGT
930 TGGCTAACTA ACCGATTGAT 1020 ATATCTCCTT	TATAGAGGAA 1110 GCTTGGTCCT CGAACCAGGA	1200 TCCCTGAAAG AGGGACTTTC	1290 TGGGTCGCAT ACCCAGCGTA	1380 AACACCCTGT TTGTGGGACA	1470 GCTTACTGGG CGAATGACCC	1560 ACCTCTGGGG TGGAGACCCC	1650 AGCGGCGTGC TCGCCGCACG	1740 ACCCAGACCT TGGGTCTGGA
100 1010 1010 1020 1030 940 950 950 960 970 980 990 970 980 990 970 980 990 970 980 990 970 980 990 970 970 970 970 970 970 970 970 97	GAACCATGGT TAAATTTAAC TATAGAGGAA TCCAGAGCTC AGAGATCTAT TGGCCAGTTA GCTAACCTTA AGAACGCCGG CGAACGATCG 1090 1100 11100 1110 1110 1120 1170 CACCATGGAG TTGTGGTTAA GCTTGGTCCT TCCTTGTCCT TGTTTTAAAA GOTGTCCAGT GTGAAGTGAA TCTGGTGGAG TCTGGGGGAGGTGAGTACCTAA GCTTGGTGGA ACAGAGGA AGAAATTTT CCACAGTCA CACTTCACTT	1180 1250 1250 1250 1250 1250 1250 1250 125	1270 1280 1280 1300 1310 1320 1320 1330 1340 1350 1350 1340 1350 1350 1350 1350 1350 1350 1350 135	1310 1420 1420 1430 TCTCCAGAAA CAGCCTCTG AAGTCTGAGG ACACAGCCAT GTATTACTGT AAGTCTCAGA CAATGCCAAA GAACCCTGT ACCTGCAAAT GAGCCGTCTG AAGTCTGAGG ACACAGCCAT GTATTACTGT AGAGGTCTC TTAGGGACA TGGAGGTTAA CTCGGCAGAC TTCAGACTCC TGTGTCGGTA CATAATGACA	1460 TOGACGACGG GCCTGGTTT ACCTGCTGCC CCGGACCAAA	1540 1550 1560 1560 1570 1580 1580 1580 1690 1690 1690 1690 1690 1690 1690 169	1630 1640 1650 1660 1650 CCCCTGACC AGCGGCGTGC ACACCTTCCC GGCTGTCCTA CCTTGAGGACGG CCGACAGGAT	1720 1730 1740 1750 1760 1800 1800 1800 1800 1800 1800 1800 18
910 TCTATATAGG AGATATATTC 1000 CTTGGTACCA	GAACCATGGT 1090 CACCATGGAG GTGGTACCTC	1180 GCTTAGTGCA CGAATCACGT	1270 CTCCAGAGAA GAGGTCTCTT	1360 TCTCCAGAGA AGAGGTCTCT	1450 TOGACGACGG ACCTGCTGCC	1540 TGGCACCCTC ACCGTGGGAG	1630 GGAACTCAGG CCPTGAGTCC	1720 TGCCCTCCAG ACGGGAGGTC

1890	1980	2070	2160	2250	2340	2430	2520	2610	2700
AGTCCAGGGC	TTTTCCCCAG	GAGCCATATC	CCAGATTCCA	CCAGGCCTCG	GCCACATGGA	CACAGGTGTA	ACATCGCCGT	TCTACAGCAA	ACACGCAGAA
TCAGGTCCCG	AAAAGGGGTC	CTCGGTATAG	GGTCTAAGGT	GGTCCGGAGC	CGGTGTACCT	GTGTCCACAT	TGTAGCGGCA	AGATGTCGTT	TGTGCGTCTT
1810 1820 1830 1830 1830 1830 1850 1850 1860 1860 1870 1880 1890 GGCCAGCACAG GGAGGGAGG GTGTCTGCTG GAAGCCAGGG TCAGCGCTCC TGCCTGGACG CATCCGGGCT ATGCAGCCCC AGTCCAGGCC CGGGTCGTGC GTAGGGCCGA TACGTCGGGG TCAGGTCCCG	1980 1950 1950 1970 1970 1980 AGCHAGGA GCCCCTTTCCCCAG TCCTTCTGGCT TTTTCCCCAG TCGTTCCCC GCCCCTTC GGAGAAGTGG GCCTCGGAG ACGGGCGG TGATACGAG TCCTCTCC AGAAAGTGG AAAAGGGGT	1990 2000 2000 2010 2020 2030 2030 2040 2050 2060 2070 GCTCGCCTA ACCCCAACACA ACCCCACAACACACACACACACA	2080 2090 2100 2110 2120 2120 2130 2140 2150 2150 CGGGAGGACC CTGCCCTGAA CCTAAAGGC CAGATTCCA ACTCCCTCAG CTGGGACGACA CTTATCTCA CTGGAGGAC GAGCTGGGAACACC TACTCTCGTG GAGGAGGAC GAGCTCTGG GACGGGAACT GGATTCGGGT GGGTTTCGG GTTTTGAGGA TGAGGAAGTC GAGCCTGTGG AAGAGAGGAG GGTCTAAGGT	2170 2180 2190 2200 2210 2220 2230 2230 2240 2250 2250 2330 2240 2250 2250 2250 2250 2250 2250 225	2250 2270 2280 2290 2290 2390 2390 2390 2390 2390 239	2350 1350 2410 2420 2430 2430 2430 2430 2430 2430 243	2410 2450 2510 2510 2520 2510 2520 2510 2520 2510 2520 252	2530 2590 2600 2600 CONTROLL C	2620 2630 2690 2700 2700 2620 2620 2620 2620 2620 2630 2630 2700 2630 2700 2700 2700 2700 2700 2700 2700 27
1870	1960	2050	2140	2230	2320	2410	2500	2590	2680
CATCCCGGCT	AGGGAGAGGG	GCTGGGCTCA	CTCGGACACC	CCGTGCCCAG	TGGGTACCAA	TACAGGGCAG	CAAAGGCTTC	CTCCGACGGC	TGAGGCTCTG
GTAGGGCCGA	TCCCTCTCCC	CGACCCGAGT	GAGCCTGTGG	GGCACGGGTC	ACCCATGGTT	ATGTCCCGTC	GTTTCCGAAG	GAGGCTGCCG	ACTCCGAGAC
1860	1950	2040	2130	2220	2310	2400	2490	2580	2670
TGCCTGGACG	ACTCATGCTC	AGGGGCAGGT	ACTCCCTCAG	CACATGCCCA	ACACACCACG	CCTCTGTCCC	CCTGCCTGGT	CCGTGCTGGA	CCGTGATGCA
ACGGACCTGC	TGAGTACGAG	TCCCCGTCCA	TGAGGGAGTC	GTGTACGGGT	TGTGTGGTGC	GGAGACAGGG	GGACGGACCA	GGCACGACCT	GCCACTACGT
1850	1940	2030	2120	2210	2300	2390	2480	2570	2660
TCAGCGCTCC	TGCCCGCCCC	CTGCACACAA	CAAACTCTCC	ACAAAACTCA	GCATCCAGGG	GCTGTACCAA	GTCAGCCTGA	ACCACGCCTC	TTCTCATGCT
AGTCGCGAGG	ACGGCGGGG	GACGTGTGTT	GTTTGAGAGG	TGFFFTGAGT	CGTAGGTCCC	CGACATGGTT	CAGTCGGACT	TGGTGCGGAG	AAGAGTACGA
1840	1930	2020	2110	2200	2290	2380	2470	2560	2650
GAAGCCAGGC	CGGAGGCCTC	AACCCAGGCC	CCCCAAAGGC	AAATCTTGIG	AGAGTAGCCT	GAGAGTGACC	CAAGAACCAG	CAACTACAAG	GGGGAACGTC
CTTCGGTCCG	GCCTCCGGAG	TTGGGTCCGG	GGGGTTTCCG	TTTAGAACAC	TCTCATCGGA	CTCTCACTGG	GFTCTTGGTC	GITGATGITC	CCCCTTGCAG
1810 1820 1830 1840 1850 1860 1860 1860 1860 CATCCCGGCT ATCCAGGCCCC CCGGTCGTCT CCCTCGGCCC CATCCCGGCT ATCCAGGCCCC CCGGTCGTCTC CCCTCCCTCC CACAGACGAC CTTCGGTCCG AGTCGCGGG ACGACCTCC GTAGGGCCCGA TACGTCGGGG	AGCAAGGCAG GCCCCTTTT CCTTTTCACC CGGAGGCCTC TGCCCGCCCC ACTCATGCTC TCGTTCCTC CGGAGGAAGTGG GCCTCCGGAG ACGGGCGGGG TGAGTACGAG	2010 AGGTGCCCT TCCACGGGBA	2100 CCTAAGCCCA GGATTCGGGT	2170 2190 2190 2200 2210 CAAACTCCCA ATCTTGTG ACAAAACTCC TAGAAAAAAAAAA	2280 CAGGTGCCCT GTCCACGGGA	2370 CCTCTGCCCT GGAGACGGGA	2460 ATGAGCTGAC TACTCGACTG	2540 2550 2560 2570 2580 AGCAATGGGC AGCCGGAGAA CAACTACAAG ACCAGGCCTC CCGTGCTGGA TCGTTACATGTTC TGGTGCGGAG GGCACGACCT	2640 GGTGGCAGCA CCACCGTCGT
1820	1910	2000	2090	2180	2270	: 2360	2450	2540	2630
GGGAGGGAGG	GCCCCGTCTG	GGCACAGGCT	CTGCCCCTGA	ATCTTCTCTC	CAAGGCGGGA	CTCGGCCCAC	CCATCCCGGG	AGCAATGGGC	GACAAGAGCA
CCCTCCCTCC	CGGGCAGAC	CCGTGTCCGA	GACGGGGACT	TAGAAGAGAG	GTTCCGCCCT	GAGCCGGGTG	GGTAGGGCCC	TCGTTACCCG	CTGTTCTCGT
1810	1900	1990	2080	2170	2260	2350	2440	2530	2620
GGCCAGCACA	AGCAAGGCAG	GCTCTGGGCA	CGGGAGGACC	GTAACTCCCA	CCCTCCAGCT	CAGAGGCCGG	CACCCTGCCC	GGAGTGGGAG	GCTCACCGTG
CCGGTCGTGT	TCGTTCCGTC	CGAGACCCGT	GCCTCCTGG	CATTGAGGGT	GGGAGGTCGA	GTCTCCGGCC	GTGGGACGGG	CCTCACCCTC	CGAGTGGCAC

2790 TGCTTGGCAC ACGAACCGTG	2880 ATGGTTCTTT TACCAAGAAA	2970 TGTGCAGGTG ACACGTCCAC	3060 AGCAGCACCT TCGTCGTGGA	3150 TTCTGTGAGC AAGACACTCG	3240 CTACCCCCAC GATGGGGGTG	1330 CCTGTGGAGG GGACACCTCC	3420 CACCACACAC GTGGTGTGTG	3510 GAACACTCCT CTTGTGAGGA	3600 TCAGACAAAC AGTCTGTTTG
2780 CGCACGAGGA GCGTGCTCCT	2870 CGAGACTGTG GCTCTGACAC	2960 TOGCCCAGGC ACCGGGTCCG	3050 CCCTCCCTCC GGGAGGGAGG	3140 GACTGTCCTG CTGACAGGAC	3230 CCTCACCCAT GGAGTGGGTA	3320 ACTCTCGGGC TGAGAGCCCG	3410 GCCACACGGC CGGTGTGCCG	3500 TCGCACACGT AGCGTGTGCA	3590 GCTGACCTGC CGACTGGACG
2770 CTCTCGCGGT GAGAGCGCCA	2860 TGGGCCCCTG ACCCGGGGAC	2950 GTCCCCACAC CAGGGGTGTG	3040 GCCAGCGTGG CGGTCGCACC	3130 CTCTGTAGGA GAGACATCCT	3220 ACAGGCCCTC TGTCCGGGAG	3310 GGGGACATGC CCCCTGTACG	3400 AGGTTGGCCG TCCAACCGGC	3490 AGCAAGGTCÇ TCGTTCCAGG	3580 ITCTCCACAT NAGAGGIGTA
GCAAGCCCCC GCTCCCCGGG CTCTCGCGGT CGCACGAGGA CGTTCGGGGG CTCTCGCGGG CGCACGAGGGCCCA GCGTGCTCCT	2840 2850 2860 2870 2880 TAAAGCACCC AGGGCTGCCC TGGGCCCCTG CGAGACTGTG ATGGTTCTTT ATTTCGTGGG TCGCGACGG ACCCGGGAAC GCTCTGACAC TACCAAGAAA	2930 2940 2950 2950 2950 GAGGCAGAGC GGGTCCCACT GTCCCCACAC TGCCCAGGGC CTCCGTCTC CCCAGGGTGA CAGGGGTGT ACCGGGTCCG	3030 TGGGGGATTT ACCCCTAAA	3110 3120 GACAGACACA CAGCCCCTGC CTGTCTGTGT GTCGGGGACG	3210 GTGCGTAGGG CACGCATCCC	3300 AACCGACTCC TTGGCTGAGG	3390 CCCCGCACTG GGGGCGTGAC	3480 CCCAGACCAG GGGTCTGGTC	3570 TCTCGGCAGC AGAGCCGTCG
2750 GCAAGCCCCC CGTTCGGGGG	2840 TAAAGCACCC ATTTCGTGGG	2930 GAGGCAGAGC CTCCGTCTCG	3020 CTCGGCAGGG GAGCCGTCCC	3110 3120 GACAGACACA CAGCCCCTGC CTGTCTGTGT GTCGGGGACG	3200 CCTAGTCCAT GGATCAGGTA	3290 ATGGGGACAC TACCCCTGTG	3380 GTTCAACAAA CAAGTTGTTT	3470 CTGCACAGCA GACGTGTCGT	3560 CCCACGAGCC GGGTGCTCGG
2740 GCGACGGCCG CGCTGCCGGC	2830 AGCATGGAAA TCGTACCTTT	2920 TGGCATGAGG ACCGTACTCC	3010 3020 3030 3030 3030 3040 3050 3050 3060 AGGGGCTGCC CTCGGCAGGG TGGGGGATTT GCCAGCGTGG CCCTCCCTCC AGCAGCACTTCCCCGACGG GAGCCGTCCC ACCCCTAAA CGGTCGCACC GGGAGGGAGG TCGTCGTGAA	3100 AGCCCCTGGG TCGGGGACCC	3190 CGGGGGCATG GCCCCCGTAC	3280 TCGCACCCGC AGCGTGGGCG	3370 3380 3390 3400 3400 3410 GCCCAGACCG GTTCAACAAA CCCCGCACTG AGGTTGGCCG GCCACACGGC CGGTCTTT GGGCGTGAC TCCAACCGGC CGGTGTCCCG	3460 CCCGGGCGAA GGGCCCGCTT	3550 CACCTCAAGG GTGGAGTTCC
2720 2730 2740 2750 2760 2760 2770 2780 2770 2780 2790 2790 2790 2790 2790 2790 2790 279	2820 CCGGGCGCCC GGCCCGCGG	2900 2910 GGCCGAGTCT CAGGCCTGAG CCGGCTCAGA CTCCGGACTC	3000 GGGCTCAGCC CCCCAAGTCGG	3080 3080 3090 GGGCCACGGG AAGCCCTAGG CCCGGTGCCC TTCGGGGATCC	3180 3190 3200 CATGCCCACT CGGGGGCATG CCTAGTCCAT GTGCGTAGGG ACAGGCCCTC GTACGGGTGA GCCCCGTAC GGATCAGGTA CACGCATCC TGTCCGGGAG	3270 CTGCCCAGCC GACGGGTCGG	3360 CACACACTCA GTGTGTGAGT	3450 GGAGCCTCA GCCTCGGAGT	3540 CCCCACGCGG 3600TGCGCC
	2830 2810 2820 2830 CTACCCCTG TACATACTTC CCGGGCGCCC AGCATGGAAACTTC CATGGGGGAC ATGTATGAAG GGCCCCCCGGG TCGTACCTTT		2980 2990 3000 3010 3020 3020 3020 3030 3050 3030 3050 305		3160 3170 3230 3230 3230 3230 3230 3230 3230 32	3250 3300 3310 3320 3320 33290 33290 3300 3300 3310 3320 3320 3320 3320 332	3340 3350 3360 3370 3380 3390 3490 3490 3400 GACTGGTGCA GATCCACACA GACTGGTCG GATCGACACA GACTGGTCG GATCGACACA GACTGGTCG GATCGACACACA GAGTTGGTCG GAGTTGTTT GAGCGTGAC TACGACCGGC GGTGTGTTT GAGCGTGAC TACGACCGGC GGTGTGCCC	3430 3490 3490 3500 3460 3470 3480 3480 3500 3500 3500 3500 3500 3500 3500 35	3520 3520 3530 3540 3550 3560 3560 3570 3580 3580 3570 3580 3590 3690 CGGACCEGGAG CCCCCACAGGGGGGGGGGGGGGGGGGG
2710 GAGCCTCTCC CTCGGAGAGG	2800 GTACCCCTG CATGGGGGAC	2890 CCACGGGTCA GGTGCCCAGT	2980 TGCCTGGGCC ACGGACCCGG	3070 GCCCTGGGCT CGGGACCCGA	3160 GCCCTGTCC CGGGACAGG	3250 GGCACTAACC CCGTGATTGG	3340 GACTGGTGCA CTGACCACGT	3430 ACACGTGCAC TGTGCACGTG	3520 CGGACACAGG GCCTGTGTCC

3690 TGGCCCACTT ACCGGGTGAA	3780 CCCGTGCCTT GGGCACGGAA	3870 CATTCTATTC GTAAGATAAG	3960 ATGGCTTCTG TACCGAAGAC	4050 GTTACGCGCA CAATGCGCGT	4140 CCTCTCAAAA GGAGAGTTTT	4230 CCCAGITICG GGGTCAAGGC	4320 AAGTAGTGAG TTCATCACTC	4400 TTGACGCAA TCCTAGCGTG AACTGCCGTT AGGATCGCAC	4500 ATTGGCAAGA TAACCGTTCT
3680 TCCCTGGCCC AGGGACCGGG	3760 3770 CATCTGTTGT TTGCCCCTCC GTAGACAACA AACGGGAGG	3860 GAGTAGGTGT CTCATCCACA	3950 3960 GGTGGGCTCT ATGGCTTCTG CCACCCGAGA TACCGAAGAC	4040 GGGTGTGGTG CCCACACCAC	4130 GTYCGCCGGG CAAGCGGCCC	4220 CCTAACTCCG GGATTGAGGC	4310 GCTATTCCAG CGATAAGGTC	4400 TTGACGGCAA AACTGCCGTT	4490 AAATATGGGG TTTATACCCC
3670 3680 CCACGTCACG TCCCTGGCCC GGTGCAGTGC AGGGACCGGG	3760 CATCTGTTGT GTAGACAACA	3840 3850 3860 GAAATTGCAT CGCATTGTCT GAGTAGGTGT CTTTAACGTA GCGTAACAGA CTCATCCACA	3940 CTGGGGATGC GACCCCTACG	AGCGGCGAT TAAGCGCGGC TCGCCGCGTA ATTCGCGCCG	4120 TTCTCGCCAC AAGAGCGGTG	4210 4220 CCAGCC CCTAACTCCG CCCAGTTCCG GGTAGGGCGG GGATTGAGGC GGGTCAAGGC	4310 CGGCCTCTGA GCTATTCCAG GCCGGAGACT CGATAAGGTC	4390 CGCGCCAAAC GCGCGGTTTG	4480 CCGTGTCCCA GGCACAGGGT
3660 GGATCACACA CCTAGTGTGT	3750 AGTTGCCAGC TCAACGGTCG		3930 AGCAGGCATG TCGTCCGTAC	4020 AGCGGCGCAT TCGCCGCGTA	4110 4120 4130 4130 4120 4130 TTCCGCCAC GTTCGCCGGG AAAGCGAAAG AAAGCGAAAG AAAGCGCGTG CAAGCGGCCC	4200 CTAACTCCGC GATTGAGGCG	4290 GAGGCCGCCT CTCCGGCGGA	4380 GCTGCGATTT CGACGCTAAA	4470 TGCATCGTCG ACGTAGCAGC
3650 CACACACAGG GTGTGTGTCC	3740 TGTGCCTTCT ACACGGAAGA	3820 3830 TCCTTTCCTA ATAVAATGAG AGGAAAGGAT TATTTTACTC	3910 GCGAGGATTG GGAAGACAAT CCCTCCTAAC CCTTCTGTTA	4000 4010 GGTATCCCCA CGCGCCTGT CCATAGGGGT GCGCGGGACA	4100 TTTCGCTTTC AAAGCGAAAG	4190 AGTCCCCCC TCAGGGCGG	4270 4310 4320 TITITATITA TICAGAGGCC GAGGCCGCCT CGGCCTCTGA GCTATTCCAG AAGTAGTGAGAAAAAAAA ACGTCTCCGG CTCCGGCGGA GCCGGAGACT CGATAAGGTC TTCATCACTC	4370 ACAGCTCAGG TGTCGAGTCC	4460 ACCATTGAAC TGGTAACTTG
3630 3640 3650 3650 3660 3650 3660 3670 3690 3690 3690 3690 3690 3690 3690 369	3730 3740 3750 3750 3750 3770 CAGCCTCGAGC TGTGCCCTTCT AGTTGCCAGC CATCTGTTGT TTGCCCTCC GTCGGAGCTG AACGGGAGG TCAACGGTCG GTAGACAACA AACGGGAGG	3820 TCCTTTCCTA AGGAAAGGAT	3910 GGGAGGATTG CCCTCCTAAC	4000 60TATCCCCA CGCGCCCTGT AGCGGCGCAT TAAGCGCGGC CCATAGGGGT GCGGGGACA TCGCCGCGTA ATTCGCGCCG	4000 4120 4130 4130 4130 4130 4130 4130 4130 413	AAGCATGCAT CTCAATTAGT CAGCAACCAT AGTCCCGCCC CTAACTCCGC TTCGTACGTA GAGTTAATCA GTCGTTGGTA TCAGGGCGGG GATTGAGGC		4330 4340 4350 4350 4360 4370 4380 4390 GAGGCTATT TGGAGGCCTA GGCTTTTGCAAAGCTTGG ACAGCTCAGG GCTGCGATT CGCGCCAAAC CTCCGAAAAA ACCTCCGGAA ACCTCCGGAAAACGT TTTTCGAACC TGTCGAGTCC CGACGCTAAA GCGCGGTTTG	4420 4430 4440 4450 4460 4460 4460 4470 4480 4480 4490 AAGGCTGGTA GGATTITATC CCGCGTGCCA AAATATGGGG TTTATATGGGG TTTATATGCCC TCCAAAATATG GGGCGACGGT AGTACCAAG TGGTAACTTG ACGTAGCAG GGCACAGGGT TTTATACCCC
3630 GTGCCCTGC CACGGGGACG	3720 CAGGACGGAT GTCCTGCCTA	3810 ACTCCCACTG TGAGGGTGAC	3900 GACAGCAAGG CTGTCGTTCC	3990 GGCTCTAGGG CCGAGATCCC	4070 4080 4080 TACACTUCC ACCCCCTAG CCCCCCCTAC ATGTGAACGG TCCCGGGGATC GCGGGCGAGG	4170 CTCAATTAGT GAGTTAATCA	4250 4250 GCCCCATGGC TGACTAATTT CGGGGTACCG ACTGATTAAA	4350 TCGAGGCCTA GCTTTTGCA ACCTCCGGAT CCGAAAACGT	4440 CCCGCTGCCA GGGCGACGGT
3620 TCTCACAAGG AGAGTGTTCC	3700 3710 CCCAGTGCCG CCCTTCCCTG GGGTCACGGC GGGAAGGGAC	3800 GGAAGGTGCC CCTTCCACGG	3890 GGTGGGGCAG CCACCCCGTC	3980 AACCAGCTGG TTGGTCGACC	4070 TACACTTGCC ATGTGAACGG	4160 AAGCATGCAT TTCGTACGTA	4240 4250 4250 4250 CCCATTCTC GCCCATGC TGACTAATTT GGGTAAGAGG CGGGGTACCG ACTGATTAAA	4340 TGGAGGCCTA ACCTCCGGAT	4430 GGATTTTATC CCTAAATAG
3610 CCAGCCCTCC GGTCGGGAGG	3700 CCCAGTGCCG GGGTCACGGC	3790 CCTTGACCCT GGAACTGGGA	3880 TOGGOGGTGG ACCCCCCACC	3970 AGGCGGAAAG TCCGCCTTTC	4060 GCGTGACCGC CGCACTGGCG	4150 AAGGGAAAA TTCCCTTTTT	4240 CCCATTCTCC GGGTAAGAGG	4330 GAGGCTTTTT CTCCGAAAAA	4420 AAGGCIGGIA TTCCGACCAT

5400 AGGACTYTYCC TCCTGAAAGG	5390 GAAGACCCCA CTTCTGGGGT	5370 5380 5380 5380 CAAAAAAGAA GAGACTTTCC GTTTTTCTT CCTTTCCAT CTTCTGGGGT TCCTGAAAGG	5370 CAAAAAAGAA GTYTYTYTYCTY	5360 TCTACTCCTC AGATGAGGAG	5320 5320 5330 5340 5340 CCCANCTAGT GAIGHTGAGG CTACTGCCTG CCCCANCTACAT TCTACTCCTC CGGTAGATCA CTACTACTCC GAIGACGAC GAGAGTTGTA AGATGAGGAG	5340 CTACTGCTGA GATGACGACT	5330 GATGATGAGG CTACTACTCC	5320 GCCATCTAGE CGGTAGATCA	
5310 CAGAAGAAAT GTCTTCTTTA	5300 CTGTTTTGCT GACAAAACGA	5290 TGAGGAAAAC ACTCCTTTTG	5280 ATGCCTTTAA TACGGAAATT	5270 CAGTGGTGGA GTCACCACCT	5210 5220 5220 5220 5220 5230 5230 5230 523	5250. ATGGAACTGA TACCTTGACT	5240 ATTCCAACCT TAAGGTTGGA	5230 TGTATTTTAG ACATAAAATC	
5220 TAATTGTFTG ATTAACAAAG	5210 CTACTGATTC GATGACTAAG	5200 ATGTGTTAAA TACACAATTT	5190 TAAGTGTATA ATTCACATAT	5180 ATAAAATTTT TATTTTAAAA	S110 5120 5210 5220 5220 5220 5220 5220 5	5160 TTTAAAGCTC AAATTTCGAG	S140 S150 GGACAAACTA CCTACAGAGA CCTGTTTGAT GGATGTCTCT	5140 GGACAAACTA CCTGTTTGAT	
5130 TGACATAATT ACTGTATTAA	5120 TTCTGTGGTG AAGACACCAC	5100 5110 5130 5130 5130 TCTTTGTGAA GGAACCTTAC TTCTGTGGTG TGACATAATT AGAAACACCAC ACTGTATTAA	5100 TCTTTGTGAA AGAAACACTT	5090 GCTTTAGATC CGAAATCTAG	5050 5110 5120 5130 5130 5130 5130 5130 5130 5130 513	5070 AGACCATGGG TCTGGTACCC	SOSO SOSO TAAAGCTATG CATTTTTATA ATTTCGATAC GTAAAAATAT	5050 TAAAGCTATG ATTTCGATAC	
5040 GCTCCCCTCC CGAGGGGAGG	5030 CAAGTTCTCT GTTCAAGAGA	4990 5000 5010 5040 5040 5040 5070 5030 5040 TIGAAGICTA CGAGAAGAAA GACTAACAGG AAGATGCTTT CAAGTTCTCT GCTCCCTCC AACTTCAGGA GTTCAAGAGA GTTCAAGAGA GAGGGGAGG	S010 GACTAACAGG CTGATTGTCC	S000 CGAGAAGAAA GCICITICITIT		4980 AAGTATAAGT TYCATATYCA	4960 4970 TCCAGGAGGA AAAAGGCATC AGGTCCTCT TTTTCCGTAG	4960 TCCAGGAGGA AGGTCCTCCT	
4950 CTCTCTGAGG GAGAGACTCC	4940 CCCAGGCGTC GGGTCCGCAG	4920 4930 4940 4950 TATAAACTIC TCCCAGAATA CCCAGGCGTC CTCTCTGAGG ATAITIGAAG AGGGTCTTAT GGGTCCGCAG GAGAACTCC		CAGAAATIGA TITGGGGAAA GICTITAACI AAACCCCITT		4890 ACGTTTTTCC TGCAAAAGG	4810 4880 TGCAGGAATT TGAAAGTGAC ACGTCCTTAA ACTTTCACTG	4870 TGCAGGAATT ACGTCCTTAA	
4860 ACAAGGATCA TGTTCCTAGT	4850 ACTCTTTGTG TGAGAAACAC	4830 4840 AATCAACCAG GCCACCTTAG TTAGTTGGTC CGGTGGAATC	4830 AATCAACCAG TTAGTTGGTC	4820 GGAAGCCATG CCTTCGGTAC	4810 4820 4830 4800 4800 4800 4820 4830 4830 TAGACATGGT TYGGAGAGCCATG AATCAACCAG GCCACCTTAGACCTAACCAACCTATCAG CCTCCGTCAA GACAAATGGT CCTTCGGTAC TYAGTTGGTC CGGGGAATC	4800 GGAGGCAGTT CCTCCGTCAA	4780 4790 TAGACATGGT TTGGATAGTC ATCTGTAGCA AACCTATCAG	4780 TAGACATGGT ATCTGTACCA	
4770 GCAAGTAAAG CGTTCATTTC	4760 ACCGGAATTG TGGCCTTAAC	4750 TTATTGAACA AATAACTTGT	4740 GCCTTAAGAC CGGAATTCTG	4730 TTTGGATGAT AAACCTACTA	4690 4750 4750 4750 4770 TCAAAGAACC ACCACGAGGA GCTCATTITC TIGCCAAAAG TITGGAIGAT GCCTIAAGAC TIAITGAACA ACGGAATIG GCAAGIAAAG AGTITCTIGG IGGIGCICCT CGAGIAAAAG AACGGIITIC AAACCIACTA CGGAAITCIG AAIAACTIGI IGGCCTIAAC CGITCAIITC	4710 GCTCATTTTC CGAGTAAAAG	4700 ACCACGAGGA TGGTGCTCCT	4690 TCAAAGAACC AGTTTCTTGG	
4680 AGTAGAGAAC TCATCTCTTG	4670 4670 TATAGTTCTC AGTAGAACATCTTG TCATCTTTG	4630 4640 4650 4660 CCATTCCTGA GARGAATCGA CCTTTAAAGG ACAGAATTAA GGTAAGGACT CTTCTTAGCT GGAAATTTCC TGTCTTAATT	4650 CCTTTAAAGG GGAAATTTCC	4640 GAAGAATCGA CTTCTTAGCT		GGGTAGGAAA ACCTGGTTCT CCCATCCTTT TGGACCAAGA	4610 GGGTAGGAAA CCCATCCTTT	4600 TGGTGATTAT ACCACTAATA	
4590 AAACAGAATC TTTGTCTTAG	4580 AGTGGAAGGT TCACCTTCCA	4570 CAACCTCTTC GTTGGAGAAG	4560 AGAATGACCA TCTTACTGGT	4550 GTACTTCCAA CATGAAGGTT	4510 4520 4580 4580 ACGAGACAGA ACGAGITCAA GIACITCCAA AGAAIGACCA CAACCICITC AGIGGAAGGI AAACAGAAIC ACGGAGACCI ACCCIGGCCI CCGCICAGGA ACGAGITCAA GIACITCCAA AGAAIGACCA CAACCICITC AGIGGAAGGI AAACAGAAIC IGCCICIGGA IGGGACCGGA GGCGAGICCI IGCICAAGII CAIGAAGGII ICITACIGGI GIIGGAGAAG ICACCIICCA ITIGICIIAG	4530 CCGCTCAGGA GGCGAGTCCT	ACGGAGACCT ACCCTGGCCT TGCCTCTGGA TGGGACCGGA	4510 ACGGAGACCT TGCCTCTGGA	

1TAATAAGGA AATTATTCCT 5760 CTCCCACACC GAGGGTGTGG 6200 6210 TTATCCGCTC ACAATTCCAC AATAGGCGAG TGTTAAGGTG 5850 AGCAATAGCA TCGTTATCGT 6280 6280 6390 FOR TCACTGCCCG AGTGTAATTA ACGCAACGCG AGTGACGGCG 6000 6010 6010 6020 6030 CCCAACTIGT TTATTGCAGC TTATAATGGT TACAAATAAA GGGTTGAACA AATAAGGTCG AATATTACCA ATGTTTATTT 5910 5910 5910 TCATCAATGT ATCTTAATGA GTCTGGATGG AGTAGTTACA TAGAATAGTA CAGACCTAGG 5560 5570 5580 TIATAAICAT AACATACIGT TITITICTTAC AATAITAGTA TIGTAIGACA AAAAAGAATG ACATTICCCC A 5740 5750 TTTTACTTGC TTTAAAAAAC AAAATGAACG AAATTTTTTG 5830 S830 S840 CTTATAATGG TTACAAATAA GAATATTACC AATGTTTATT CITITITAATT 1 6190 TGTGAAATTG ACACTTTAAC GCCATAACAG 5730 TTTGTAGAGG 1 TTGTCCAAAC 6180 CTGTTTCCTG GTGAGCTNAC 5630 5640 CAAAAATIGT GTACCTITAG GTTTTTAACA CATGGAAATC 5800 S810 S820 CAATTOTIGT TGFTAACTIG TTTAFTGCAG GTTAACAACA ACAATTGAAC AAATAACGTC 6260 TGCCTAATGA (CTTCGCCCAC CAAGCGGGTG C 5720 CCATACCACA GGTATGGTGT ACTGCATTCT TGACGTAAGA TAGTTGTGGT ATGGTCATAG 5530 5540 TTCTGTAACC TTTATAAGTA AAGACATTGG AAATATTCAT 5620 TAACTATGCT (S890 CACTGCATTC 1 GTGACGTAAG A 5980 TGCTGGAGTT (ACGACCTCAA (5710 TCATAATCAG AGTATTAGTC CATITITIC CATANANA CANANANA TGGCGTAATC ACCGCATTAG AGCCGGNAGC ATANAGUCTA AAGCCTGGGG TCGGCCTTCG TATTTCACAT TTCGGACCCC 5520 TGGAAAATA CTGCTATTAA TGACTAGAGA AAAATGAATG TTTTACTTAC SSSO GCATTTTTT CGTAAAAAA 5430 CTAAGTITIT TGAGTCATGC GATTCAAAAA ACTCAGTACG 5950 5960 5970 GCTGGATGAT CCTCCAGGGC GGGGATCTCA CGACCTACTA GGAGGTCGCG CCCCTAGAGT CACAAATITIC ACAAATAAAG GTGTTTAAAG TGTTTATTTC AGCTAGAGCT TCGATCTCGA 5600 CATAGAGIGE (GTATCTCACA (S780 CCTGAAACAT 2 CGACTTTGTA 5510 AAGAAAATTA TTCTTTTAAT 5680 5680 ATATTYGATG TATAGTGCCT TATAAACTAC ATATCACGGA GTCGACCTCT TCACAAATTT CACAAATAAA AGIGITTAAA CIGITTAITT 6220 ACAACATACG 7 TGTTGTATGC 1 GCAATAGCAT (CGTTATCGTA (TCTGTATACC (5770 TCCCCCTGAA AGGGGGACTT TTCAGAATTG 5500 ACTGCTATAC TGACGATATG 5590 TCCACACAGG AGGTGTGTCC

6390 CGCTCTTCCG GCGAGAAGGC	6480 TTATCCACAG AATAGGTGTC	6570 SCGTTTTTCC SGCAAAAGG	6660 PACCAGGCGT ATGGTCCGCA	6750 SGAAGCGTGG CCTTCGCACC	6840 CCCGTTCAGC GGGCAAGTCG	6930 ACTGGTAACA TGACCATTGT	7020 GTATTTGGTA CATAAACCAT	7110 GGTGGTTTTT CCACCAAAAA	7200 CAGTGGAACG GTCACCTTGC	
6330 6330 6340 6350 6350 6350 6330 6330 6330 6330 7CGTGCCAGC TGCATTAATG AATCGCCCAA CGCGCGGGA GAGGCGGTTT GCGTATTGGG CGCTCTTCCG AGCACGGTC ACGTAATTAC TTAGCCGGTT GCGCGCCCT CTCCGCCAAA CGCATAAACC GCGAGAAGGC	6470 GGTAATACGG 1 CCATTATGCC 7	6510 6510 6510 6520 6530 6530 6540 6550 6550 6550 6550 6550 6550 655	6600 6610 6620 6620 6630 6630 6630 6640 6650 6640 6650 6660 6660 6650 6660 6650 6660 6650 6660 6650 6660 6650 6660 6650 6660 6650 6	6750 6730 6730 6730 6730 6730 6730 6730 673	6840 6810 6830 6830 6840 6840 6840 6840 6820 6830 6830 6830 6840 6840 6840 6840 6840 6840 6840 684	CCGACCGCTC CGCCTTATCC GGTAACTATC GTCTTGAGTC GAACCGGTA AGACACGACT TATCGCCACT GGCAGCAGCC ACTGGTAACA CCGACCGCTC GGCCTTATCC GGTAACTATC GTCTTGAGTC CAACCGGTA TCTGTGCTGA ATAGCGGTGA CCOTCGTCGG TGACCATTGT	6980 6990 7000 7010 7020 6980 6980 6980 6980 6980 6980 6980 7000 7010 7020 6980 6980 6980 7010 7020 7020 7020 7020 7020 7020 702	7010 7030 7040 7050 7060 7060 7070 7070 7080 7090 7100 7100 7010 7030 7090 7100 7110 7090 7090 7100 7110 7090 709	710 7190 7190 7200 7120 7130 7140 7150 7160 7160 7170 7170 TIGITICA GARCHANT ACCOCHAN ANANAGAIC TCARGNAGAI CCTITGAICT TITCTACGG GICTGACGT CAGIGGAACG AACAACGII CGICGICINA TGCGCGICIT TITITCCING AGTICIICIA GANACIAGA ANGAIGCC CAGACIGGA GICACCITGC	
GAGGCGGTTT GCGTATTGGG CTCCGCCAAA CGCATAACCC	6470 6480 6410 6420 6430 6440 6430 6440 6450 6460 6460 6470 CITCCTCGCT CACTGACTCG CTGCGCTCGG TCGTTCGGCTC GCGCGCGGGCG GTATCAGCTC ACTCAAGGC GGTATAGGC GAAAAAAAAAA	6550 GTAAAAAGGC CATTTEFCCG	6640 ACCCGACAGG TGGGCTGTCC	6730 TCTCCGCCTT ACAGGCGGAA	6820 TGGGCTGTGT ACCCGACACA	6910 TATCGCCACT ATAGCGGTGA	7000 ACGCCTACAC TGCCCATGTG	7040 7050 7050 7060 7060 7000 GCTGAAGCCA CGCTGGTAGC GCTGAAGCCA CATACCAC CGCTGGTAGC CGACTICG CATACTAGCC CGCTGTAGC CGACTICGG CATATICTCA ACCATGGAGA ACTAGGCCGT TIGITIGGTG GCGACCATCG	7180 TTTCTACGGG AAAGATGCCC	
6360 CGCGCGGGGA GCGCGCCCT	6450 GTATCAGCTC CATAGTCGAG	6540 GCCAGGAACC CGGTCCTTOG	6630 AGGTGGCGAA TCCACCGCTT	6720 ACCGGATACC TGGCCTATGG	6810 CGCTCCAAGC GCGAGGTTCG	6900 AGACACGACT TCTGTGCTGA	6990 TGGCCTAACT ACCGGATTGA	TGATCCGGCA ACTAGGCCGT	1170 CCTTTGATCT GGAAACTAGA	
6340 6350 TGCATTAATG AATCGCCCAA ACGTAATTAC TTAGCCGGTT	6440 GCGGCGAGCG CGCCGCTCGC	6530 CCAGCAAAA GGTCGTTTTTC	6620 CTCAAGTCAG GAGTTCAGTC	6710 CCTGCCGCTT GGACGCCGAA	6800 GTAGGTCGTT CATCCAGCAA	6890 CAACCCGGTA GTTGGGCCCAT	6980 CTTGAAGTGG GAACTTCACC	1070 TGGTAGCTCT ACCATCGAGA	7160 TCAAGAAGAT AGTTCTTCTA	
6340 TGCATTAATG ACGTAATTAC	6430 TCGTTCGGCT AGCAAGCCGA	6520 GAGCAAAAGG CTCGTTTTCC	6610 AAAATCGACG TTTTAGCTGC	6700 CTGTTCCGAC GACAAGGCTG	6790 TCAGTTCGGT AGTCAAGCCA	6880 GTCTTGAGTC CAGAACTCAG	.6970 CTACAGAGTT GATGTCTCAA	7060 GAAAAAGAGT CTTTFTCTCA	7150 AAAAAGGATC TTTTTCCTAG	
6330 TCGTGCCAGC AGCACGGTCG	6420 6410 6420 6420 crrcrccrc caccacaca cabarabaca aroacacaca aroacacaca caccacacacacacacacacacacacacaca	6510 AAGAACATGT TTCTTGTACA	6580 6590 6600 6600 6610 6620 6620 ANGGETECH GACCATCACA AAAATCGACG CCCCCTGAC GACCATCACA AAAATCGACG CTCAAGGCAAGG	6690 OTCCCCTCTC CACCCCAGAG	6760 6770 6780 6790 6890 6890 CGCTITCTCA ATGCTCACGC TGTAGGTATC TCAGTITCCGT GTAGGTCGTT GCTAAAGAC TACCAGGAA ACATCCATAG AGTCAAGCCA CATCCAGCAA	6870 GGTAACTATC CCATTGATAG	6960 GTAGGCGGTG CATCCGCCAC	7050 GTTACCTTCG CAATGGAAGC	7140 ACCCCAGAA TGCCCCTCTT	
6320 GGGAAACCTG CCCTTTGGAC	6410 CACTGACTCG GTGACTGAGC	6500 TAACGCAGGA	6590 CCCCCTGAC	6680 AAGCTCCCTC	6770 ATGCTCACGC TACGAGTGCG	6860 CGCCTTATCC GCGGAATAGG	6950 AGCGAGGTAT TCGCTCCATA	7040 GCTGAAGCCA CGACTTCGGT	7130 GCAGCAGATT CGTCGTCTAA	
6310 CTTCCAGTC GAAAGGTCAG	6400 CTTCCTCGCT	6490 AATCAGGGGA	6580 ATAGGCTCCG	6670 TTCCCCCTGG	6760 CGCTTTCTCA	6850 CCGACCGCTG	6940 GGATTAGCAG	7030 TCTGCGCTCT	7120 TTGTTTGCAA AACAAACGTT	

TTTAAATCAA AAATTTAGTT	7380 TITCGTICAT AAAGCAAGTA	7470 CCGCGAGACC GGCGCTCTGG	7560 TCCGCCTCCA AGGCGGAGGT	7650 ACAGGCATCO TGTCCGTAGC	7740 TTGTGCAAAA AACACGTTTT	7830 CTGCATAATT GACGTATTAA	7920 CGGCGACCGA GCCGCTGGCT	8010 TCTTCGGGGC AGAAGCCCCG	8100 TTTACTTTCA AAATGAAAGT
7280 AAAATGAAGT TTTTACTTCA	7370 7380 GATCTGTCTA TTTCGTTCAT CTAGACAGAT AAAGCAAGTA	7460 TGCAATGATA ACGTTACTAT	7550 7550 7550 7550 7550 7550 7550 7550	7650 7650 7650 7650 7650 7650 ANIMOTITIGE GCAACGITIGE TGCCATIGET ACAGGCATCG TIAICAAACG CGITIGCAACA ACGGTAACGA TGICCGIAGG	7730 ATCCCCCATG TAGGGGGTAC		7850 7910 7920 7920 7920 7920 7920 7920 7920 792	930 7940 7950 8000 7950 900 900 900 900 900 900 900 9000 90	8100 8030 8090 8050 8060 8070 8080 8090 8100 8080 8090 8100 GAAAACTCTC AAGGATCTTA CCGCTGTTGA GATCAGTTC GATGTAACCC ACTCGTGCAC CCAACTGATC TTCAGCATCT TTTACTTTCA
AAGGATCTTC ACCTAGATCC TTTTAAATTA TTCCTAGAAG TGGATCTAGG AAAATTTAAT	7360 CTATCTCAGC GATAGAGTCG	7450 GCCCCAGTGC CGGGGTCACG	AGCCGGAAGG GCCGAGCGCA GAAGTGGTCC TCGGCCTTCC CGGCTCGCGT CTTCACCAGG	7630 GCAACGTTGT CGTTGCAACA	CGGTTCCCAA CGATCAAGGC GAGTTACATG GCCAAGGGTT GCTAGTTCCG CTCAATGTAC	7810 CACTCATGGT GTGAGTACCA	7900 CATTCTGAGA GTAAGACTCT	7990 TGCTCATCAT ACGAGTAGTA	8080 CCAACTGATC GGTTGACTAG
AAGGATCTTC ACCTAGATCC TTCCTAGAAG TGGATCTAGG	7350 AGTGAGGCAC TCACTCCGTG	7440 TTACCATCTG AATGGTAGAC	7530 GCCGAGCGCA CGGCTCGCGT		7710 CGATCAAGGC GCTAGTTCCG	7800 GCAGTGTTAT CGTCACAATA	7890 TCAACCAAGT AGTTGGTTCA	7980 ACTTTAAAAG TGAAATTTTC	8070 ACTCGTGCAC TGAGCACGTG
7250 AAGGATCTTC TTCCTAGAAG	7340 ATGCTTAATC TACGAATTAG	7430 ACGGGAGGGC TGCCCTCCCG	7520 AGCCGGAAGG TCGGCCTTCC	7610 TTCGCCAGTT AAGCGGTCAA	7700 CGGTTCCCAA GCCAAGGGTT	7790 TAAGTTGGCC ATTCAACCGG	7880 TGGTGAGTAC ACCACTCATG	7970 ACATAGCAGA TGTATCGTCT	8060 GATGTAACCC CTACATTGGG
7240 GATTATCAAA CTAATAGTTT	ACTIGGICIG ACAGITACCA AIGCITAAIC AGIGAGGCAC CIAICICAGG IGAACCAGA TOTCAAIGGI TAGGAAITAG ICACICCGIG GAIAGAGICG	7410 7420 7430 7440 GPCGTGTAGA TAACTACGAT ACGCGAGGGC TTACCATCTG CAGCACATCT ATTGATGCTA TGCCCTCCCG AATGGTAGAC	7510 TAAACCAGCC ATTTGGTCGG	CGGGAAGCIA GAGTAAGTAG GCCCTTCGAT CTCATTCATC	7690 CATTCAGCTC GTAAGTCGAG	7780 TTGTCAGAAG AACAGTCTTC	7870 TITICIGIGAC AAAGACACIG	7960 ATACCGCGCC TATGGCGCGG	8050 GATCCAGTTC CTAGGTCAAG
7230 TTGGTCATGA AACCAGTACT	7320 ACTTGGTCTG TGAACCAGAC	7410 GTCGIGTAGA CAGCACATCT	7510 GGCTCCAGAT TTATCAGCAA TAAACCAGCC CCGAGGTCTA AATAGTCGTT ATTTGGTCGG	7590 CGGGAAGCTA GCCCTTCGAT	7680 GGTATGGCTT CCATACCGAA	CCTCCGATCG	7860 GTAAGATGCT CATTCTACGA	CCCGGCGTCA ATACGGGATA ATACCGCGCC ACATAGCAGA ACTITAAAAG GGGCGGCAGT TATGGCGCGG TGTATCGTCT TGAAATTTTC	8040 CCGCTGTTGA GCCGACAACT
7210 7210 7220 7230 7240 AAAACTCAGG TTAAGGGATF TTGGTCATGA GATTATCAAA TTTTGAGTGC AATTCCCTAA AACCAGTAGT CTAATAGTTT	7310 ATATGAGTAA TATACTCATT	7400 CTGACTCCCC GACTGAGGGG	7490 GGCTCCAGAT CCGAGGTCTA	7510 7520 7580 7590 7600 7600 7600 7610 7CCAGTCTA TAATTGTTGC CGGGAAGCTA GAGTAAGTAG TTCGCCAGTT AGGTCAGATA ATTAACAAGG GCCCTTCGAT CTCATTCATC AAGCGGTCAA	7720 7770 7770 7770 7770 7770 7770 7770	7750 7750 7760 7770 7770 77780 7790 7800 7800 7810 7820 7820 7820 7820 7820 7820 7820 782	7840 7850 7850 7860 7870 CTCTTACTOT CATCCCATCC GTAAGATGCT TITCTGTGAC GAGAATGACA AAAGACACTG	7940 CCCGGCGTCA GGGCCGCAGT	8020 8030 8040 8050 GAAAACTCTC AAGGATCTTA CCGCTGTTCA GATCCAGTTC CTTTTGAGAG TTCCTAGAAT GGCGACAACT CTAGGTCAAG
7210 AAAACTCACG TTTTGAGTGC	7300 TCTAAAGTAT AGATTTCATA	7390 CCATAGITGC GGTATCAACG	7480 CACGCTCACC	7570 TCCAGTCTAT AGGTCAGATA	7660 TGGTGTCACG ACCACAGTGC	7750 AAGCGGTTAG TTCGCCAATC	7840 CTCTTACTGT GAGAATGACA	7930 GTTGCTCTTG CAACGAGAAC	8020 GAAAACTCTC CTTTTGAGAG

8190 CTCATACTCT GAGTATGAGA	8280 AAACAAATAG TTTGTTTATC	
8180 ATGTTGAATA TACAACTTAT	8270 TTAGAAAAT AATCTTTTTA	
8170 CGACACGGAA GCTGTGCCTT	8260 TTGAATGTAT AACTTACATA	
8160 GGAATAAGGG CCTTATTCCC	8250 GGATACATAT CCTATGTATA	
8150 CGCAAAAAG GCGTTTTTTC	8240 TCTCATGAGC AGAGTACTCG	8330 G
8110 8120 8130 8140 8150 8160 8170 8180 8180 8190 8190 8190 8190 8190 819	8200 8210 8220 8230 8240 8250 8250 8270 8270 8280 8280 8280 8280 8280 828	8320 8320 8320 8320 8320 8320 8320 8320
8130 AAAACAGGAA TTTTGTCCTT	8220 AGCATTTATC TCGTAAATAG	8310 CGAAAAGTGC GCTTTTCACG
8120 TGGGTGAGCA ACCCACTCGT	8210 ATATTATTGA TATAATAACT	8300 CACATTTCCC GTGTAAAGGG
8110 CCAGCGTTTC GGTCGCAAAG	8200 TCCTTTTTCA AGGAAAAGT	8290 GGGTTCCGCG CCCAAGGCGC

Comparison of whole chiBR96 and deleted CH2 chiBR96 on Ley/K ELISA

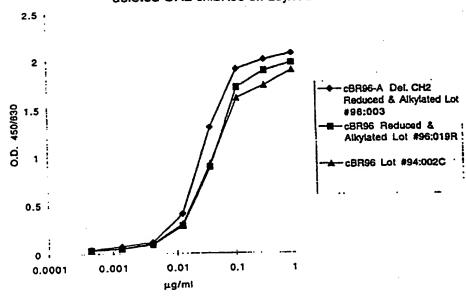


Figure 15

hBR96-2B: L235 to A235 and G237 to A237

hBR96-2C: E318 to S318, K320 to S320, and K322 to S322

hBR96-2D: P331 to A331

hBR96-2E: L235 to A235, G237 to A237, E318 to S318, K320 to S320, and K322 to S322

hBR96-2F: L235 to A235, G237 to A237, and P331 to A331

hBR96-2G: E318 to S318, K320 to S320, K322 to S322, and P331 to A331

hBR96-2H: L235 to A235, G237 to A237, E318 to S318, K320 to S320, K322 to S322, and P331 to A331 $\,$

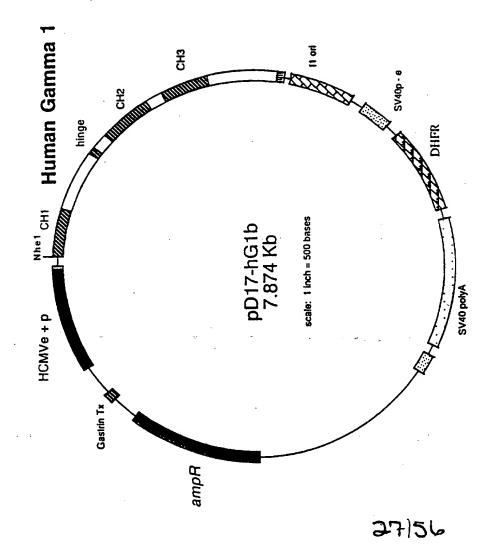


FIGURE 17

FIGURE 18A

1	GGTACCAATT	TAAATTGATA	TCTCCTTAGG	TCTCGAGTCT	CTAGATAACC
51	GGTCAATCGA	TTGGAATTCT	TGCGGCCGCT	TGCTAGCCAC	CATGGAGTTG
101	TGGTTAAGCT	TGGTCTTCCT	TGTCCTTGTT	TTAAAAGGTG	TCCAGTGTGA
151	AGTGCAACTG	GTGGAGTCTG	GGGGAGGCTT	AGTGCAGCCT	GGAGGGTCCC
201	TGCGACTTTC	CTGTGCTGCA	TCTGGATTCC	CGTTCAGTGA	CTATTACATG
251	TATTGGGTTC	GCCAGGCTCC	AGGCAAGGGA	CTGGAGTGGG	TCTCATACAT
301	TAGTCAAGAT	GGTGATATAA	CCGACTATGC	AGACTCCGTA	AAGGGTCGAT
351	TCACCATCTC	CAGAGACAAT	GCAAAGAACA	GCCTGTACCT	GCAAATGAAC
401	AGCCTGAGGG	ACGAGGACAC	AGCCGTGTAT	TACTGTGCAA	GAGGCCTGGC
451	GGACGGGGCC	TGGTTTGCTT	ACTGGGGCCA	AGGGACTCTG	GTCACGGTCT
501	CTTCCGCTAG	CACCAAGGGC	CCATCGGTCT	TCCCCCTGGC	ACCCTCCTCC
551	AAGAGCACCT	CTGGGGGCAC	AGCGGCCCTG	GGCTGCCTGG	TCAAGGACTA
601	CTTCCCCGAA	CCGGTGACGG	TGTCGTGGAA	CTCAGGCGCC	CTGACCAGCG
551	GCGTGCACAC	CTTCCCGGCT	GTCCTACAGT	CCTCAGGACT	CTACTCCCTC
701	AGCAGCGTGG	TCACCGTGCC	CTCCAGCAGC	TTGGGCACCC	AGACCTACAT
751	CTGCAACGTG	AATCACAAGC	CCAGCAACAC	CAAGGTGGAC	AAGAÄAGTTG
801	GTGAGAGGCC	AGCACAGGGA	GGGAGGGTGT	CTGCTGGAAG	CCAGGCTCAG
851	CGCTCCTGCC	TGGACGCATC	CCGGCTATGC	AGCCCCAGTC	CAGGGCAGCA
901	AGGCAGGCCC	CGTCTGCCTC	TTCACCCGGA	GGCCTCTGCC	CGCCCCACTC
951	ATGCTCAGGG	AGAGGGTCTT	CTGGCTTTTT	CCCCAGGCTC	TGGGCAGGCA
1001	CAGGCTAGGT	GCCCCTAACC	CAGGCCCTGC	ACACAAAGGG	GCAGGTGCTG
1051	GGCTCAGACC	TGCCAAGAGC	CATATCCGGG	AGGACCCTGC	CCCTGACCTA
1101	AGCCCACCCC	AAAGGCCAAA	CTCTCCACTC	CCTCAGCTCG	GACACCTTCT
1151	CTCCTCCCAG	ATTCCAGTAA	CTCCCAATCT	TCTCTCTGCA	GAGCCCAAAT
1201	CTTGTGACAA	AACTCACACA	TGCCCACCGT	GCCCAGGTAA	GCCAGCCCAG
1251	GCCTCGCCCT	CCAGCTCAAG	GCGGGACAGG	TGCCCTAGAG	TAGCCTGCAT
1301	CCAGGGACAG	GCCCCAGCCG	GGTGCTGACA	CGTCCACCTC	CATCTCTTCC

1351	TCAGCACCTG	235 AACTCETEG	A37 CEGACCGTCA	GTCTTCCTCT	TCCCCCCÁAA
1401	ACCCAAGGAC	ACCCTCATGA	TCTCCCGGAC	CCCTGAGGTC	ACATGCGTGG
1451	TGGTGGACGT	GAGCCACGAA	GACCCTGAGG	TCAAGTTCAA	CTGGTACGTG
1501	GACGGCGTGG	AGGTGCATAA	TGCCAAGACA	AAGCCGCGGG	AGGAGCAGTA
1551	CAACAGCACG	TACCGTGTGG		CACCGTCCTG	CACCAGGACT
1601	GGCTGAATGG	CAAGGAGTAC	AAGTGCAAGG	TCTCCAACAA	AGCCCTCCCA
1651	GCCCCATCG	AGAAAACCAT	CTCCAAAGCC	AAAGGTGGGA	CCCGTGGGGT
1701	GCGAGGGCCA	CATGGACAGA	GGCCGGCTCG	GCCCACCCTC	TGCCCTGAGA
1751	GTGACCGCTG	TACCAACCTC	TGTCCCTACA	GGGCAGCCCC	GAGAACCACA
1801	GGTGTACACC	CTGCCCCCAT	CCCGGGATGA	GCTGACCAAG	AACCAGGTCA
1851	GCCTGACCTG	CCTGGTCAAA	GĠCTTCTATC	CCAGCGACAT	CGCCGTGGAG
1901	TGGGAGAGCA	ATGGGCAGCC	GGAGAACAAC	TACAAGACCA	CGCCTCCCGT
1951	GCTGGACTCC	GACGGCTCCT	TCTTCCTCTA	CAGCAAGCTC	ACCGTGGACA
2001	AGAGCAGGTG	GCAGCAGGGG	AACGTCTTCT	CATGCTCCGT	GATGCATGAG
2051	GCTCTGCACA	ACCACTACAC	GCAGAAGAGC	CTCTCCCTGT	CTCCGGGTAA
2101	ATGAGTGCGA	CGGCCGGCAA	. GCCCCCGCTC	CCCGGGCTCT	CGCGGTCGCA
2151	CGAGGATGCT	TGGCACGTAC	CCCCTGTACA	TACTTCCCGG	GCGCCCAGCA
2201	TGGAAATAAA	GCACCCAGCG	CTGCCCTGGG	CCCCTGCGAG	ACTGTGATGG
2251	TTCTTTCCAC	GGGTCAGGCC	GAGTCTGAGG	CCTGAGTGGC	ATGAGGGAGG
2301	CAGAGCGGGT	CCCACTGTCC	CCACACTGG	CCAGGCTGTC	CAGGTGTGCC
2351	TGGGCCCCCI	AGGGTGGGG	TCAGCCAGG	GCTGCCCTCC	GCAGGGTGGG
2401	GGATTTGCC	CCGTGGCCCT	CCCTCCAGC	A GCACCTGCC	TGGGCTGGGC
2451	CACGGGAAG	CCTAGGAGC	C CCTGGGGAC	A GACACACAG	CCCTGCCTCT
2501	GTAGGAGAC	GTCCTGTTC	T GTGAGCGCC	C CTGTCCTCC	C GACCTCCATG
2551	CCCACTCGG	GGCATGCCT	A GTCCATGTG	C GTAGGGACA	G GCCCTCCCTC
2601	ACCCATCTA	CCCCACGGC	A CTAACCCCT	G GCTGCCCTG	C CCAGCCTCGC
2651	ACCCGCATG	G GGACACAAC	C GACTCCGGG	G ACATGCACT	C TCGGGCCCTG
2701	TGGAGGGAC	r GGTGCAGAT	G CCCACACAC	A CACTCAGCC	C AGACCCGTTC
2751	AACAAACCC	C GCACTGAGG	T TGGCCGGCC	A CACGGCCAC	C ACACACACAC
2801	GTGCACGCC	T CACACACGG	A GCCTCACCC	G GGCGAACTG	C ACAGCACCCA
					29156

2851	GACCAGAGCA	AGGICCTCGC	ACACGTGAAC	ACTCCTCGGA	CACAGGCCCC
2901	CACGAGCCCC	ACGCGGCACC	TCAAGGCCCA	CGAGCCTCTC	GGCAGCTTCT
2951	CCACATGCTG	ACCTGCTCAG	ACAAACCCAG	CCCTCCTCTC	ACAAGGGTGC
3001	CCCTGCAGCC	GCCACACACA	CACAGGGGAT	CACACACCAC	GTCACGTCCC
3051	TGGCCCTGGC	CCACTTCCCA	GTGCCGCCCT	TCCCTGCAGG	ACGGATCAGC
3101	CTCGACTGTG	CCTTCTAGTT	GCCAGCCATC	TGTTGTTTGC	CCCTCCCCCG
3151	TGCCTTCCTT	GACCCTGGAA	GGTGCCACTC	CCACTGTCCT	TTCCTAATAA
3201	AATGAGGAAA	TTGCATCGCA	TTGTCTGAGT	AGGTGTCATT	CTATTCTGGG
3251	GGGTGGGGTG	GGGCAGGACA	GCAAGGGGGA	GGATTGGGAA	GACAATAGCA
3301	GGCATGCTGG	GGATGCGGTG	GGCTCTATGG	CTTCTGAGGC	GGAAAGAACC
3351	AGCTGGGGCT	CTAGGGGGTA	TCCCCACGCG	CCCTGTAGCG	GCGCATTAAG
3401	CGCGGCGGGT	GTGGTGGTTA	CGCGCAGCGT	GACCGCTACA	CTTGCCAGCG
3451	CCCTAGCGCC	CGCTCCTTTC	GCTTTCTTCC	CTTCCTTTCT	CGCCACGTTC
3501	GCCGGGCCTC	TCAAAAAAGG	GAAAAAAAGC	ATGCATCTCA	ATTAGTCAGC
3551	AACCATAGTC	CCGCCCTAA	CTCCGCCCAT	CCCGCCCCTA	ACTCCGCCCA
3601	GTTCCGCCCA	TTCTCCGCCC	CATGGCTGAC	TAATTTTTTT	TATTTATGCA
3651	GAGGCCGAGG	CCGCCTCGGC	CTCTGAGCTA	TTCCAGAAGT	AGTGAGGAGG
3701	CTTTTTTGGA	GGCCTAGGCT	TTTGCAAAAA	GCTTGGACAG	CTCAGGGCTG
3751	CGATTTCGCG	CCAAACTTGA	CGGCAATCCT	AGCGTGAAGG	CTGGTAGGAT
3801	TTTATCCCCG	CTGCCATCAT	GGTTCGACCA	TTGAACTGCA	TCGTCGCCGT
3851	GTCCCAAAAT	ATGGGGATTG	GCAAGAACGG	AGACCTACCC	TGGCCTCCGC
3901	TCAGGAACGA	GTTCAAGTAC	TTCCAAAGAA	TGACCACAAC	CTCTTCAGTG
3951	GAAGGTAAAC	AGAATCTGGT	GATTATGGGT	AGGAAAACCT	GGTTCTCCAT
4001	TCCTGAGAAG	AATCGACCTT	TAAAGGACAG	AATTAATATA	GTTCTCAGTA
4051	GAGAACTCAA	AGAACCACCA	CGAGGAGCTC	ATTTTCTTGC	CAAAAGTTTG
4101	GATGATGCCT	TAAGACTTAT	TGAACAACCG	GAATTGGCAA	GTAAAGTAGA
4151	CATGGTTTGG	ATAGTCGGAG	GCAGTTCTGT	TTACCAGGAA	GCCATGAATC
4201	AACCAGGCCA	CCTTAGACTC	TTTGTGACAA	GGATCATGCA	GGAATTTGAA
4251	AGTGACACGT	TTTTCCCAGA	AATTGATTTG	GGGAAATATA	AACTTCTCCC
4301	AGAATACCCA	GGCGTCCTCT	CTGAGGTCCA	GGAGGAAAAA	GGCATCAAGT

30/56

4351	ATAAGTTTGA	AGTCTACGAG	AAGAAAGACT	AACAGGAAGA	TGCTTTCAAG
4401	TTCTCTGCTC	CCCTCCTAAA	GCTATGCATT	TTTATAAGAC	CATGGGACTT
4451	TIGCTGGCTI	TAGATCTCTT	TGTGAAGGAA	CCTTACTTCT	GTGGTGTGAC
4501	ATAATTGGAC	AAACTACCTA	CAGAGATTTA	AAGCTCTAAG	GTAAATATAA
4551	AATTTTTAAG	TGTATAATGT	GTTAAACTAC	TGATTCTAAT	TGTTTGTGTA
4601	TTTTAGATTC	CAACCTATGG	AACTGATGAA	TGGGAGCAGT	GGTGGAATGC
4651	CTTTAATGAG	GAAAACCTGT	TTTGCTCAGA	AGAAATGCCA	TCTAGTGATG
4701	ATGAGGCTAC	TGCTGACTCT	CAACATTCTA	CTCCTCCAAA	AAAGAAGAGA
4751	AAGGTAGAAG	ACCCCAAGGA	CTTTCCTTCA	GAATTGCŢAA	GTTTTTTGAG
4801	TCATGCTGTG	TTTAGTAATA	GAACTCTTGC	TTGCTTTGCT	ATTTACACCA
4851	CAAAGGAAAA	AGCTGCACTG	CTATACAAGA	AAATTATGGA	AAAATATTCT
4901	GTAACCTTTA	TAAGTAGGCA	TAACAGTTAT	AATCATAACA	TACTGTTTTT
4951	TCTTACTCCA	CACAGGCATA	GAGTGTCTGC	TATTAATAAC	TATGCTCAAA
5001	AATTGTGTAC	CTTTAGCTTT	TTAATTTGTA	AAGGGGTTAA	TAAGGAATAT
5051	TTGATGTATA	GTGCCTTGAC	TAGAGATCAT	AATCAGCCAT	ACCACATTTG
5101	TAGAGGTTTT	ACTTGCTTTA	AAAAACCTCC	CACACCTCCC	CCTGAACCTG
5151	AAACATAAAA	TGAATGCAAT	TGTTGTTGTT	AACTTGTTTA	TTGCAGCTTA
5201	TAATGGTTAC	AAATAAAGCA	ATAGCATCAC	AAATTTCACA	AATAAAGCAT
5251	TTTTTTCACT	GCATTCTAGT	TGTGGTTTGT	CCAAACTCAT	CAATGTATCT
5301	TATCATGTCT	GGATCGGCTG	GATGATCCTC	CAGCGCGGG	ATCTCATGCT
5351	GGAGTTCTTC	GCCCACCCCA	ACTTGTTTAT	TGCAGCTTAT	AATGGTTACA
5401	AATAAAGCAA	TAGCATCACA	AATTTCACAA	ATAAAGCATT	TTTTTCACTG
5451	CATTCTAGTT	GTGGTTTGTC	CAAACTCATC	AATGTATCTT	ATCATGTCTG
5501		ACCTCTAGCT	AGAGCTTGGC	GTAATCATGG	TCATAGCTGT
5551	TTCCTGTGTG	AAATTGTTAT	CCGCTCACAA	TTCCACACAA	CATACGAGCC
5601	GGAAGCATAA	AGTGTAAAGC	CTGGGGTGCC	TAATGAGTGA	GCTAACTCAC
5651	ATTAATTGCG	TTGCGCTCAC	TGCCCGCTTT	CCAGTCGGGA	AACCTGTCGT
5701	GCCAGCTGCA	TTAATGAATC	GGCCAACGCG	CGGGGAGAGG	CGGTTTGCGT
5751	ATTGGGCGCT	CTTCCGCTTC	CTCGCTCACT	GACTCGCTGC	GCTCGGTCGT
5801	TCGGCTGCGG	CGAGCGGTAT	CAGCTCACTC	AAAGGCGGTA	ATACGGTTAT

31/56

5351	CCACAGAATC	AGGGGATAAC	GCAGGAAAGA	ACATGTGAGC	AAAAGGCCAG
5901	CAAAAGGCCA	GGAACCGTAA	AAAGGCCGCG	TTGCTGGCGT	TTTTCCATAG
5951	GCTCCGCCCC	CCTGACGAGC	ATCACAAAAA	TCGACGCTCA	AGTCAGAGGT
6001	GGCGAAACCC	GACAGGACTA	TAAAGATACC	AGGCGTTTCC	CCCTGGAAGC
6051	TCCCTCGTGC	GCTCTCCTGT	TCCGACCCTG	CCGCTTACCG	GATACCTGTC
6101	CGCCTTTCTC	CCTTCGGGAA	GCGTGGCGCT	TTCTCAATGC	TCACGCTGTA
6151	GGTATCTCAG	TTCGGTGTAG	GTCGTTCGCT	CCAAGCTGGG	CTGTGTGCAC
6201	GAACCCCCCG	TTCAGCCCGA	CCGCTGCGCC	TTATCCGGTA	ACTATCGTCT
6251	TGAGTCCAAC	CCGGTAAGAC	ACGACTTATC	GCCACTGGCA	GCAGCCACTG
6301	GTAACAGGAT	TAGCAGAGCG	AGGTATGTAG	GCGGTGCTAC	AGAGTTCTTG
6351	AAGTGGTGGC	CTAACTACGG	CTACACTAGA	AGGACAGTAT	TTGGTATCTG
6401	CGCTCTGCTG	AAGCCAGTTA	CCTTCGGAAA	AAGAGTTGGT	AGCTCTTGAT
6451	CCGGCAAACA	AACCACCGCT	GGTAGCGGTG	GTTTTTTTGT	TTGCAAGCAG
6501	CAGATTACGC	GCAGAAAAA	AGGATCTCAA	GAAGATCCTT	TGATCTTTTC
6551	TACGGGGTCT	GACGCTCAGT	GGAACGAAAA	CTCACGTTAA	GGGATTTTGG
6601	TCATGAGATT	ATCAAAAAGG	ATCTTCACCT	AGATCCTTTT	AAATTAAAA
6651	TGAAGTTTTA	AATCAATCTA	AAGTATATAT	GAGTAAACTT	GGTCTGACAG
5701	TTACCAATGC	TTAATCAGTG	AGGCACCTAT	CTCAGCGATC	TGTCTATTTC
6751	GTTCATCCAT	AGTTGCCTGA	CTCCCCGTCG	TGTAGATAAC	TACGATACGG
6801	GAGGGCTTAC	CATCTGGCCC	CAGTGCTGCA	ATGATACCGC	GAGACCCACG
6851	CTCACCGGCT	CCAGATTTAT	CAGCAATAAA	CCAGCCAGCC	GGAAGGGCCG
6901	AGCGCAGAAG	TGGTCCTGCA	ACTTTATCCG	CCTCCATCCA	GTCTATTAAT
6951	TGTTGCCGGG	AAGCTAGAGT	AAGTAGTTCG	CCAGTTAATA	GTTTGCGCAA
7001	CGTTGTTGCC	ATTGCTACAG	GCATCGTGGT	GTCACGCTCG	TCGTTTGGTA
7051	TGGCTTCATT	CAGCTCCGGT	TCCCAACGAT	CAAGGCGAGT	TACATGATCC
7101	CCCATGTTGT	GCAAAAAAGC	GGTTAGCTCC	TTCGGTCCTC	CGATCGTTGT
7151	CAGAAGTAAG	TTGGCCGCAG	TGTTATCACT	CATGGTTATG	GCAGCACTGC
7201	ATAATTCTCT	TACTGTCATG	CCATCCGTAA	GATGCTTTTC	TGTGACTGGT
7251	GAGTACTCAA	CCAAGTCATT	CTGAGAATAG	TGTATGCGGC	GACCGAGTTG
7301	CTCTTGCCCG	GCGTCAATAC	GGGATAATAC	CGCGCCACAT	AGCAGAACTT

32/56

7351 TAAAAGTGCT CATCATTGGA AAACGTTCTT CGGGGCGAAA ACTCTCAAGG 7401 ATCTTACCGC TGTTGAGATC CAGTTCGATG TAACCCACTC GTGCACCCAA 7451 CTGATCTTCA GCATCTTTTA CTTTCACCAG CGTTTCTGGG TGAGCAAAAA 7501 CAGGAAGGCA AAATGCCGCA AAAAAGGGAA TAAGGGCGAC ACGGAAATGT 7551 TGAATACTCA TACTCTTCCT TTTTCAATAT TATTGAAGCA TTTATCAGGG 7601 TTATTGTCTC ATGAGCGGAT ACATATTTGA ATGTATTTAG AAAAATAAAC 7651 AAATAGGGGT TCCGCGCACA TTTCCCCGAA AAGTGCCACC TGACGTCGAC 7701 GGATCGGGAG ATCTGCTAGG TGACCTGAGG CGCGCCGGCT TCGAATAGCC 7751 AGAGTAACCT TTTTTTTTAA TTTTATTTTTA TTTTATTTTT GAGATGGAGT 7801 TTGGCGCCGA TCTCCCGATC CCCTATGGTC GACTCTCAGT ACAATCTGCT 7851 CTGATGCCGC ATAGTTAAGC CAGTATCTGC TCCCTGCTTG TGTGTTGGAG 7901 GTCGCTGAGT AGTGCGCGAG CAAAATTTAA GCTACAACAA GGCAAGGCTT 7951 GACCGACAAT TGCATGAAGA ATCTGCTTAG GGTTAGGCGT TTTGCGCTGC 8001 TTCGCGATGT ACGGGCCAGA TATACGCGTT GACATTGATT ATTGACTAGT 8051 TATTAATAGT AATCAATTAC GGGGTCATTA GTTCATAGCC CATATATGGA 8101 GTTCCGCGTT ACATAACTTA CGGTAAATGG CCCGCCTGGC TGACCGCCCA 8151 ACGACCCCG CCCATTGACG TCAATAATGA CGTATGTTCC CATAGTAACG 8201 CCAATAGGGA CTTTCCATTG ACGTCAATGG GTGGACTATT TACGGTAAAC 8251 TGCCCACTTG GCAGTACATC AAGTGTATCA TATGCCAAGT ACGCCCCCTA 8301 TTGACGTCAA TGACGGTAAA TGGCCCGCCT GGCATTATGC CCAGTACATG 8351 ACCTTATGGG ACTTTCCTAC TTGGCAGTAC ATCTACGTAT TAGTCATCGC 8401 TATTACCATG GTGATGCGGT TTTGGCAGTA CATCAATGGG CGTGGATAGC 8451 GGTTTGACTC ACGGGGATTT CCAAGTCTCC ACCCCATTGA CGTCAATGGG 8501 AGTTTGTTTT GGCACCAAAA TCAACGGGAC TTTCCAAAAT GTCGTAACAA 8551 CTCCGCCCA TTGACGCAAA TGGGCGGTAG GCGTGTACGG TGGGAGGTCT 8601 ATATAAGCAG AGCTCTCTGG CTAACTAGAG AACCCACTGC TTACTGGCTT 8551 ATCGAAATTA ATACGACTCA CTATAGGGAG ACCCAAGCTT

FIGURE 18F

-	
G	
ڃ	
•	
1	
-	
\circ	

۵

60 GGTCAATCGA CCAGTTAGCT 120 CCTGGCACCC GGACCGTGGG	180 GGACTACTTC CCTGATGAAG	240 GCACACCTTC CGTGTGGAAG	300 CG/IGCCC/ICC GCACGGGAGG	360 CAACACCAAG GTTGTGGTTC	420 TGGAAGCCAG ACCTTCGGTC	480 GCAGCAAGGC CGTCGTTCCG	540 TCAGGGAGAG AGTCCCTCTC	600 CTAACCCAGG GATIGGGTCC
50 CTAGATAACC GATCTATTGG 110 CGGTCTTCCC GCCAGAAGGG	GGGCACAGCG GCCCTGGGCT GCCTGGTCAA CCCGTGTCGC CGGGACCCCGA CGGACCAGTT	220 230 GGCGCCCTGA CCAGCGGCGT CCGCGGGACT GGTCGCCGCA	290 GCGTGGTCAC CGCACCAGTG	350 ACAAGCCCAG TGTTCGGGTC	410 420 GGGTGTCTGC TGGAAGCCAG CCCACAGACG ACCTTCGGTC	470 CCAGTCCAGG GGTCAGGTCC	530 540 CCACTCATGC TCAGGGAGAG GGTGAGTACG AGTCCCTCTC	S60 570 580 590 CTTTTTCCCC AGGCTCTGGG CAGGCACAGG CTAGGTGCCC GAAAAAAGGGG TCCGAGACCC GTCCGTGTCC GATCCACGGG
TCTCGAGTCT AGAGCTCAGA 100 AAGGGCCCAT TTCCCGGGTA	160 GCCCTGGGCT CGGGACCCGA		280 TCCCTCAGCA AGGGAGTCGT	340 AACGTGAATC TTGCAC1TAG	400 CAGGGAGGGA GTCCCTCCCT	460 CTATGCAGCC GATACGTCGG	520 TCTGCCCGCC AGACGGGCGG	580 CAGGCACAGG GTCCGTGTCC
30 TCTCCTTAGG AGAGGAATCC 90 TGCTAGCACC ACGATCGTGG	150 GGGCACAGCG CCCGTGTCGC	210 GTGGAACTCA CACCTTGAGT	270 AGGACTCTAC TCCTGAGATG	330 CTACATCTGC GATGTAGACG	390 GAGGCCAGCA CTCCGGTCGT	450 CGCATCCCGG GCGTAGGGCC	510 520 520 CCCGGAGGCC TCTGCCCGCC GGGCCTCCGG AGACGGGCGGG	570 AGGCTCTGGG TCCGAGACCC
10 20 30 40 GGTACCAATT TAAATTGATA TCTCCTTAGG TCTCGAGTCT CCATGGTTAA ATTTAACTAT AGAGGAATCC AGAGCTCAGA 70 80 90 100 TTGGAATTCT TGCGGCCGCT TGCTAGCACC AAGGGCCCAT AACCTTAAGA ACGCCGGCGA ACGATCGTGG TTCCCGGGGTA	140 GCACCTCTGG CGTGGAGACC	200 TGACGGTGTC ACTGCCACAG	260 TACAGTCCTC ATGTCAGGAG	320 GCACCCAGAC CGTGGGTCTG	380 AAGTTGGTGA 'I'TCAACCAC'I	440 CCTGCCTGGA GGACGGACCT	500 TGCCTCTTCA ACGGAGAAGT	S60 570 580 CTTTTTCCCC AGGCTCTGGG CAGGCACAGG GAAAAAGGGG TCCGAGACCC GTCCGTGTCC
10 GGTACCAATT CCATGGTTAA 70 TTGGAATTICT AACCTFAAGA	130 TCCTCCAAGA AGGAGGTTCT	190 CCCGAACCGG GGGCTTGGCC	250 CCGGC/IOTCC GGCCGACAGG	310 AGCAGCTTGG TCGTCGAACC	370 GIGGACAAGA CACCTG1TCT	430 GCTCAGCGCT CGAGTCGCGA	490 AGGCCCCGTC TCCGGGGCAG	550 GGTCTTCTGG CCAGAAGACC
					3	ص15		

CORE 15

AAGAGCCATA TCCGGGAGGA

CAGACCTGCC

640

GCGTGGTGGT GGACGTGAGG CACGAAGACC CTGAGGTCAA GTTCAACTGG TACGTGGACG CGCACCACCA CCTGCACTCG GTGCTTCTGG GACTCCAGTT CAAGTTGACC ATGCACCTGC 910 923 950.23 950.23 950 CTGACACTC CACCTCCAGT CCGTCAGTCT CTGGGGGA CCGTCAGTCT GTGGGGGGA CCGTCAGTCT GGACTCCTCAGTC GTGGACGTAG AGAAGGAGTC GTGGACTTGA QGACTCCCTT GGCAGTCAGA GCAGTACAAC AGCACGTACC CGCACCTICCA CGTATTACGG TYCTGTTYCG GCGCCCTCCT CGTCATGTYG YCGTGCATGG 860 890 900
GACAGGTGCC CTAGAGTAGC CTGCCATCCAG GGACAGGCCC CAGCCGGGTG
CTGTCCACGG GATCTCATCG GACGTAGGTC CCTGTCCGGG GTCGGCCCAC CCGGACCCCT GAGGTCACAT AGGAGAAGGG GGGTTTTYGGG 11TCCTGTGGG AGTACTAUAG GGCCTGGGGA CTCCAGTGTA CCAAATCTTG GTTAGAAGAG AGACGTCTCG GGTTTAGAAC CGCCCTCCAG CGGGTCCGGA GCGGGAGGTC 670 680 720 720 720 720 CCCTGCCCCT GACCTARGCC CACCCCAAAG GCCAAACTCT CCACTCCCTC AGCTCGGACACACTCT CACTCCCTC AGCTCGGACGCGCA CTGGATCGG GTGGGGTTTC CGGTTTGAGA GGTGAGGGAG TCGAGCCTGT GGGACG'IG'IG T'ITCCCCGIC CACGACCCGA GICTGGACGG ITCTCGGIAI AGGCCCTCCI 1150 1160 1170 1180 1190 CTCTGCTACCACC AGGACTGGCT GAATGGCAAG CACACCTCCTCACCCA CCTTGACCCA CTTTACCGTTC 790 830 830 TGACAAAACT CACATGCC CACCGTGCCC AGGTAAGCCA GCCCAGGCCT ACTGT*TTTGA GTGTGTACGG GTGGCACGGG TCCATTCGGT CGGGTCCGGA 1010 CAATCITICITC ICTGCAGAGC CGCGGGAGGA TCCTCTTCCC CCCAAACCC AAGGACACCC TCATGATCTC 1000 760 CCGTGGAGGT GCATAATGCC AAGACAAAGC 1110 1050 CCTTCTCTCC TCCCAGATIC CAGTAACTCC GGAAGAGG AGGGTCTAAG GTCATTGAGG GTGCTGGGCT CCCTGCACAC AAAGGGGCAG GAGTTCCGCC 850 CTCAAGGCGG 35/56

FIGURE 19B

1230 23/2 1240 1250 1260 CTCCCAGCCC CCATCGAGAA AACCATCTCC AAAGCCAAAGGGAGGTCGGG GGFAGCTCTT TTGGTAGAGG TTTCGGTTTC

312 1210 1220 CACAAAGCC CATTCCAGAG GITGITTCGG

GTGGGACCCG TGGGGTGCGA CACCCTGGGC ACCCCACGCT

1270

1340 1350 1360 1370 1380 CCGCTGTACC AACCTCTGTC CCTACAGGGC AGCCCCGAGA ACCACAGGTG GGCGACA/YGG TTGGAGACAG GGATGTCCCG TCGGGGCTCT TGGTGTCCAC

CTGAGAGTGA GACTCTCACT

1330

TCTATCCCAG CGACATCGCC GTGGAGTGGG AGAGCAATGG GCAGCCGGAG AGATAGGCTC GCTGTAGCGG CACCTCACCC TCTCGTTACC CGTCGGCCTC GACCTGCCTG CCTACTCGAC TGGTTCTTGG TCCAGTCGGA CTGGACGGAC AAGCTCACCG TGGACAAGAG CAGGTGGCAG CAGGGGAACG ICTTCTCATG CTCCGTGATG GGCTCTCGCG GTCGCACGAG GATGCTTGGC CCGAGAGCGC CAGCGTGCTC CTACGAACCG 1550 1560 GCTCCTTCTT CCTCTACAGC CGAGGAAGAA GGAGATGTCG ACGTACCCCC TGTACATACT TCCCGGGCGC CCAGCATGGA AATAAAGCAC CCAGCGCTGC TCCATGGGGG ACATGTATGA AGGGCCCGCG GGTCGTACCT 'FTATTTCGTG GGTCGCGACG TICGAGINGC ACCIGITYON GICCACCOIC GICCCCTICC AGAAGAGIAC GAGGCACTAC CATGAGGCTC TGCACAACCA CTACAGGCAG AAGAGCCTCT CCCTGTCTCC GGGTAAATGA GTACTCCGAG ACGTGTTGGT GATGTGCGTC TTCTCGGAGA GGGACAGAGG CCCATTTACT 1780 1780 1800 CCAGCATGGA AATAAAGCAC CCAGCGCTGC 1430 AGGTCAGCCT 1490 1610 1670 CCCCATCCCG GGATGAGCTG ACCAAGAACC GGGGTAGGGC CCTACTCGAC TGGTTCTTGG AGACCACGCC TCCCGTGCTG GACTCCGACG TCTGGTGCGG AGGGCACGAC CTGAGGCTGC 1660 1420 1480 1600 1720 1540 1530 TCCCGTGCTG CCGCTCCCCG GGCGAGGGGC 1410 1470 1650 1710 CGGCAAGCCC 1520 AGACCACGCC 1700 CCCCTTCGGG 1400 1460 TACACCCTGC (ATGTGGGACG (GTGCGACGGC CACGCTGCCG GTCAAAGGCT AACAACTACA 1690 1390 1450 CAGTTTCCGA 1570 1630

36/56

FIGURE 19C

CTATICECTITC TCAGGCGGAA AGAACCAGCT GGGGCTCTAG GGGGTATCCC CACGCGCCCT GATACCGAAG ACTCCGCCTT TCTTGGTCGA CCCCGAGATC CCCCATAGGG GTGCGCGGGA GTAGCUGCGC ATTAAGCGCG GCGGGTGTGG TGGTTACGCG CAGCGTGACC GCTACACTTG CATCGCCGCG TAATTCGCGC CGCCCACACC ACCAATGCGC GTCGCACTGG CGATGTGAAC AGTGGCATGA GGGAGGCAGA GCGGGTCCCA CTGTCCCCAC ACTGGCCCAG GCTGTGCAGG TCACCGTACT CCCTCCGTCT CGCCCAGGGT GACAGGGGTG TGACCGGGTC CGACACGTCC GGTGGGGGAT THECCAGOGT GGCCCTCCCT CCAGCAGCAC CTGCCCTGGG CTGGGCCACG GGAAGCCCTA GCGCCCCTGT CCTCCCGACC TCCATGCCCA CTCGGGGGCA TGCTGGGGAT GCGTGGGCT CGCGGGGACA GGAGGGCTGG AGGTACGGGT GAGCCCCCGT ACGACCCCTA CGCCACCCGA CAGGCCGAGT CTGAGGCCTG GGACCCGGGG ACGCTCTGAC ACTACCAAGA AAGGTGCCCA GTCCGGCTCA GACTCCGGAC ACCCCGAGIC GGICCCCGAC GGGAGCCGIC CCACCCCIA CCCTCGGCAG TENCCTOGG CCCCCTAGGG TGGGGCTCAG CCAGGGGCTG TTCCACGGGT CCTGGGCCCC TGCGAGACIG TGATGGTTCT ACACGGACCC GGGGGATCCC

FICURE 19D

37/56

CCAGCGCCCT AGCGCCCGCT

GCTTTCCCCG TCAAGCTCTA AATCGGGGCA TCCCTTTAGG GTTCCGATTT AGTGCTTTAC CGAAAGGGGC AGTTCGAGAT TTAGCCCCGT AGGGAAATCC CAAGGCTAAA TCACGAAATG

2520	2580	2640	2700	2760	2820	2880	2940	3000
GGACTCTTGT	TAAGGGATTT	AACGCGAATT	CAGGCAGGCA	CTAACTCCGC	TGACTAAITIT	AAGTAGTGAG	GCTGCGATTT	CCCGCTGCCA
CCTGAGAACA	ATTCCCTAAA	TTGCGCTTAA	GTCCGTCCGT	GATTGAGGCG	ACTGATTAAA	TYCATCACTC	CGACGCTAAA	GCCCGACGGT
2510 CTTTAATAGT GAAATTATCA	2570 TPTTGATFTA AAAACTAAAT	2630 ACAAAAATTT TGTTTTTAAA	2690 CCAGGCTCCC GGTCCGAGGG	2750 AGTCCCGCCC TCAGGGCGGG	2810 GCCCCATGGC CGGGGTACCG			2990 GGATTTTATC CCINAAAING
2500	2560	2620	2680	2740	2800	2860	2920	2980
AGTCCACGTT	CGGTCTATTC	AGCTGATTTA	TGGAAAGTCC	CAGCAACCAT	CCCATTCTCC	CGGCCTCTGA	AAAAGCTIGG	AAGGCTGGTA
TCAGGTGCAA	GCCAGATAAG	TCGACTAAAT	ACCTTTCAGG	GTCGTTGGTA	GGGTAAGAGG	GCCGGAGACT	TITITGAACC	TTCCGACCA'F
2490	2550	2610	2670	2730	2790	2850	2910	2960 2970
TTGACGTTGG	AACCCTATCT	TTAAAAAATG	AGTTAGGGTG	CTCAATTAGT	CCCAGTTCCG	GAGGCCGCCT	GGCTTTTTGCA	TTGACGCCAA TCCTAGCGTG
AACTGCAACC	TTGGGATAGA	AATTTTTTAC	TCAATCCCAC	GAGTTAATCA	GGGTCAAGGC	CTCCGGCGGA	CCGAAAACGT	AACTGCCGTT AGGATCGCAC
2480 TTTTCGCCCT AAAAGCGGGA	2540 AACAACACTC TTGTTGTGAG	2600 GGCCTATTGG CCGGATAACC	2660 AATGTGTGTC TYACACAGG		2780 CCTAACTCCG GGATTGAGGC	2840 TGCAGAGGCC ACGTCTCCGG	2900 TGGAGGCCTA ACCTCCGGAT	
2470	2530	2590	2650	2710	2770	2830	() 2890	2950
GATAGACGGT	TCCAAACTGG	TGGGGATTTC	AATTCTGTGG	GAAGTATGCA	CCATCCGCC	U TTTTTATTTA	6 GAGGCTTTTT	CGCGCCAAAC
CTATCTGCCA	AGGTYTGACC	ACCCCTAAAG	TTAAGACACC	CTTCATACGT	GGTAGGGCGG	O AAAATAAAT	CTCCGAAAAA	GCGCGCIIITIYS
	2480 2510 TTTTCGCCCT TTGACGTYGG AGTCCACGTY CTTTAATAGT GGACTC AAAAGCGGGA AACTGCAACC TCAGGTGCAA GAAATTATCA CCTGAG	TTTTCGCCCT TTGACGTTGG AGTCCACGTT CTTTAATAGT GGACTCT AAAAGCGGGA AACTGCAACC TCAGGTGCAA GAAATTATCA CCTGAGI 2540 2550 2560 2570 AACAACACTC AACCCTATCT CGGTCTATTC TTTTGATTTA TAAGGGI	TTTTCGCCCT TTGACGTTGG AGTCCACGTT CTTTAATAGT GGACTCTAAAAGCGGGA AACTGCAACC TCAGGTGCAA GAAATTATCA CCTGAGTAACACACCT CAGGTGCAA GAAATTATCA CCTGAGTAACACACTC AACCACTTC CGCTCTATTC TTTTGATTTA TAAGGGTATTGTTGTTGTTGAGTTAAA GCCAGATAAG AAAACTAAAT TTTCCCCTGGGTAAAAATG AGCTGATTTA ACAAAAATTTTTTAC TCGACTAAAT TGTTTTTTAAAAATTC TCGACTAAAAT TGTTTTTTAAAAATTC TCGACTAAAATTTTTTAC TCGACTAAAAT TGTTTTTTAAAAATTTTTAC TCGACTAAAAT TGTTTTTTAAAAATTTTTTAC TCGACTAAAAT TGTTTTTTAAAAATTTTTAC TCGACTAAAAT TGTTTTTTAAA TTGCGCT	TTTTCGCCT TTGACGTTGG AGTCCACGTT CTTTAATAGT GGACTCT AAAAGCGGGA AACTGCAACC TCAGGTGCAA GAAATTATCA CCTGAG3 AACAACACCTC TTGACGTTGG AGTCACGTT CAAAATTATCA CCTGAG3 TTGTTGTGGA TAGGATAGA GCCAGATAAG AAAACTAAAT TAAGGG3 TTGTTGTGGA TAAAAAATG GCCAGATATA ACAAAAATTT AACGCG3 CCGGATAACC AATTTTTTTAC TCGACTAAAT TGTTTTTTAAA TTGCGCTGCTGAGGG GGTCCGAGGGG GTCCGAGGGG GTCCGTGAGTG TTAACACACAC ACCTTTCAGG GGTCCGAGGG GTCCGTG	TTTTCGCCCT TTGACGTTGG AGTCCACGTT CTTTAATAGT GGACTCT AAAAGCGGGA AACTGCAACC TCAGGTGCAA GAAATTATCA CCTGAGT 2540 2550 2560 2570 AACAACACTC AACACACTC AACCCTATCT CGCTCTATTC TTTTGATTTA TAAGGGTGTTGTTGTTGTTGTTGTTTAATAGT ACAAAATT ATTCCCCTGGGTTGGTTTTGTTTT	TTTTCGCCT TTGACGTTGG AGTCCACGTT CTTTAATAGT GGACTCT AAAAGCGGGA AACTGCAACC TCAGGTGCAA GAAATTATCA CCTGAGT TTGATGTG AACTGCAACC TCAGGTGCAA GAAATTATCA CCTGAGTTTGATTTGA	2470 2480 2590 2510 CTATCGCCT TYGACGTAGG TYGACTAGGAACC TYGACGTAGGAACC TYGACGTAGGAACC CTATCGCCA AAAAGCGGGA AACTGCAACC TYGAGGATAGC CTGAGGATAGC 1CCAAACTGG AACAACACTC AACCCTATTC TYTTGATTTA TAAAAATTA 1CCAAACTGGGATAGG CGGTCTATTC TYTTGATTTA TAAACTGAAAT 1CCAAACTGGGATAGG TYGGGATAAGG CCGCGATAAT TAAACTGAAAT 1CCAAACTGGGATAGG TYTTGATTTA TYTTGATTTA TYTCCCTAAACT 1CCAATCGTAGG TYTTAAAAAATTTA TYTTGAAAATTTA TYTCCCTAAAAATTTA TYTCCCTAAAAATTTA 1CAATTTTTTAC TYTTTTTAAAAAAAATTA TYTTTTTAAAAAAATTAAAAAAATTA TYTTTTTAAAAAAATTAAAAAAATTAAAAAAAAAAAAA	2470 2480 2490 2500 2510 CTATCTGCCA AAAAGCGGGA AACTGCAACC TCAGGTGCAA CTTTAATAGT GAAATTATCA CTGAGAATTATCA CTGAGAATTATCA CTGAGAATTATCA CTGAGAATTATCA CTGAGAATTATA CTGAGATTATA CTGAGATTATA CTGAGATTATA CTGAGATTATA CTGAGATTATA AAAAAATTATATA CTGAGATTATA AAAAAATTATATAA AAAAAATTATATAA AAAAAATTATATAA AAAAAAATTATAAA AAAAAAATTATAAA AAAAAAATTAAAAAAAAAAAAAAAAAAAAAAAAAAAA

CIGITITACCA GGAAGCCATG AATCAACCAG GCCACCITAG ACTCTTTGTG ACAAGGATCA GACAAATGGT CCTTCGGTAC ITAGTTGGTC CGGTGGAATC TGAGAAACAC TGTTCCTAGT 3420 CCATICCIGA GAAGAATCGA CCTTTAAAGG ACAGAATTAA TATAGTICTC AGTAGAGAAC GGTAAGGACT CTICTTAGCT GGAAATTICC TGTCTTAATT ATATCAAGAG TCATCTTIG 3260 3290 3300 ACCACAGAGA GCTCATTTTC TTGCCAAAG TTTGGATGAT GCCTTAAGAC TGGTGCTCCT CGAGTAAAAG AACGGTTTTC AAACCTACTA CGGAATTCTG TTATTGAACA ACCEGAATTG GCAAGTAAAG TAGACATGGT TTGGATAGTC GGAGGCAGTT AATAACTTGT TGGCCTTAAC CGTTCATTTC ATCTGTACCA AACCTATCAG CCTCCGTCAA TCATGGTTCG ACCATTGAAC TGCATCGTCG CCGTGTCCCA AAATATGGGG ATTGGCAAGA AGTACCAAGC TGGTAACTTG ACGTAGCAGC GGCACAGGGT TTTATACCCC TAACCGTTCT ACGGAGACCT ACCCTGGCCT CCGCTCAGGA ACGAGTTCAA GTACTTCCAA AGAATGACCA TGCCTCTGGA TGGGACCGGA GGCGAGTCCT TGCTCAAGTT CATGAAGGTT TCTTACTGGT AGTGGAAGGT AAACAGAATC TGGTGATTAT GGGTAGGAAA ACCTGGTTCT TCACCTTCCA TTTGTCTTAG ACCACTAATA CCCATCCTTT TGGACCAAGA 3110 3120 GTACTTCCAA AGAATGACCA 3410 3350 3170 3340 3400 3160 3390 3330 3150 3210 3090 3030 3250 3260 TCAAAGAACC ACCACGAGGA 3380 3320 3140 3200 3080 AGITTICITGG CAACCTCTTC GTTGGAGAAG 3130

FIGURE 19F

29156

TTGAAGTCTA CGAGAAGAAA GACTAACAGG AAGATGCTTT CAAGTTCTCT GCTCCCTCC AACTTCAGAAA GTTCAAGAGA CGAGGGGAGG

3570

3560

TCCCAGAATA CCCAGGCGTC CTCTCTGAGG TCCAGGAGGA AAAAGGCATC AAGTATAAGT AGGGTCTTAT GGGTCCGCAG GAGAGACTCC AGGTCCTCT TTTTCCGTAG TTCATATTCA

3520

TCTTTGI AGAAACH AAATTTGI ATTAACH ATTAACH TACGGAI TACGGAI TCCTGAI TCCTGAI TCCTGAI TTTTTTCI AAAAAGI	
3650 GCTTTAGATC CGAAATCTAG 3710 CCTACAGAGA GGATGTCTC 3770 CTACTGATTC GATGAGG GATGATGAGG GTCACCCC 3890 GATGATGAGG CTACTGGGGG TATTCTGGGGGT TTATCTTGAATTA 4010 AATAGAAATTA TTCTTTTAAT TTCTTTTTAAT TTCTTTTTAAT TTCTTTTTAAT TTCTTTTTAAT	4200 GTACCTTTAG CATGGAAATC
	4190 CAAAAATTGT GTTTTTAACA
ACTTTTG GGACAAA CCTGITT TACACAA TGAAATGG ACTTACC GGGTAGA GAGAAAG TGTGTTT ACTGCTY TGTGTTT ACTGCTY TGTGTTY ACTGCTY	4170 4180 CTGCTATTAA TAACTATGCT GACGATAATT ATTGATACGA
3630 AGACCATGGG TCTGGTACCC 3690 TGACATAATT ACTGTATAA 3750 TAAGTGTATA 3810 ATGGAACTGA TACCTTGACT 3870 CAGAAGAAAT GTCTTTTTCT 3930 TGAGTCATGC ACTCATATA 3930 TGAGACATGC ACTCATATA 4050 AAAAAGCTGC ACTCATACGACG ACTCATACTCC ACTCATACACG ACTCATACACG ACTCATACACG ACTCATACACG ACTCATACTCC ACT	
TAAAGCTATG CATTITITATA ATTITCGATAC GTAAAAATAT 3670 3680 GGAACCTTAC TICTGIGGTG CCTTGGAATG AAGACACCAC 3730 3740 TAAGGTAAAT TATTITAAAA TCCATTITAG ATTICCAACCT ACATAAAATTTT ACTTTTTAG ATTICCAACCT ACATAAAATC TAAGGTTGGA 3850 3860 TGAGGAAAAC CTGTTTTGCT ACTTTTTG ACTTACTCCTC GAGGAAAAC TCTACTCCTC GAGGAAAC TCTACTCCTC GAGGAAATT GACTAAGTTTTT AAGTCTTAAC GATTCAAAAA 4030 4100 TTCTGTAAACTTTTC AAGACATTGC ACCACAAAGGA AAGACATTGC TATATAAAGTA AAGACATTGG AATATTCAT	4160 CATAGAGTGT GTATCTCACA
3610 TAAAGCTATG 3670 GGAACCTTAC CCTTGGAATG 3730 TAAGGTAAAAT ATTCCATTTA 3150 TGAGGAAAAC ACTCCTTTTG 3910 TGAGGAAAAC ACTCCTTTTG A030 TGCTATTTAC A030 TGCTATTTAC A030 TGCTATTTAC A030 TTCTGTAAAATG A030 TTCTGTAAAATG A030	4150 TCCACACAGG AGGTGTGTCCC
40156	

4260	4320	4380	4440	4500	4560	4620	4680	4740	4800
TGACTAGAGA	CTCCCACACC	TYTYATYGCAG	GCATTTTTTT	GTCTGGATCG	CCCAACTIGE	ACAAATAAAG	TCTTATCATG	CTGTTTCCTG	ATAAAGTGTA
ACTGATCTCT	GAGGGTGTGG	AAATAACGTC	CGTAAAAAA	CAGACCTAGC	GGGTTGAACA	TGTTTATTTC	AGAATAGTAC	GACAAAGGAC	TAITTCACAT
4250	4310	4370	4430	4490	4550	4610	4670	4730	4790
TATAGEGCCT	TTTAAAAAAC	TGTTAACTTG	CACAAATAAA	ATCTTATCAT	CTTCGCCCAC	CACAAATTTC	CATCAATGTA	ATGGTCATAG	AGCCGGAAGC
ATATCACGGA	AAATTTTTG	ACAATTGAAC	GTGTTTATTT	TAGAATAGTA	GAAGCGGGTG	GTGTTTAAAG	GTAGTTACAT	TACCAGTATC	TCGGCCTTCG
4240	4300	4360	4420	4480	4540	4600	4660	4720	4780
ATATTTGATG	TTTTACTTGC	CAATTGTTGT	TCACAAATTT	TCATCAATGT	TGCTGGAGTT	GCAATAGCAT	TGTCCAAACT	TGGCGTAATC	ACAACATACG
TATAAACTAC	AAAATGAACG	GTTAACAACA	AGTGTTTAAA	AGTAGTTACA	ACGACCTCAA	CGTTATCGTA	ACAGGTTTGA	ACCGCATTAG	TGTTGTATCC
4230	4290	4350	4410	4470	4530	4590	4650	4710	4770
TTAATAAGGA	TTTGTAGAGG	AAAATGAATG	AGCAATAGCA	TTGTCCAAAC	GGGGATCTCA	TACAAATAAA	AGTTGTGGTT	AGCTAGAGCT	ACAATTCCAC
AATTATTCCT	AAACATCTCC	TTTTACTTAC	TCGTTATCGT	AACAGGTTTG	CCCCTAGAGT	ATGTTTAITT	TCAACACCAA	TCGATCTCGA	TGTTAAGGTG
4220	4280	4340	4400	4460	4520	4580	4640	4700	4760
TGTAAAGGGG	CCATACCACA	CCTGAAACAT	TTACAAATAA	TAGTTGTGGT	CCTCCAGCGC	TTATAATGGT	ACTGCATTCT	GTCGACCTCT	1TATCCGCTC
ACATTTCCCC	GGTATGGTGT	GGACTTTGTA	AATGTTTATT	ATCAACACCA	GGAGGTCGCG	AATATTACCA	TGACGTAAGA	CAGCTGGAGA	AATAGGCGAG
4210	4270	4330	4390	4450	4510	4570	4630	4690	4750
CTTTTTYAATT	TCATAATCAG	TCCCCCTGAA	CTTATAATGG	CACTGCATTC	GCTGGATGAT	TTATTGCAGC	CATTITITE	TCTGTATACC	TGTGAAATTG
GAAAAATTAA	AGTATTAGTC	AGGGGGACTT	GAATATTACC	GTGACGTAAG	CGACCTACTA	AATAACGTCG	GIRAAAAAAG	AGACATATGG	ACACTTFAAC

FIGURE 19H

4850

4840

AAGCCIVGGG TGCCTAATGA GIGAGCTAAC TCACATTAAT TGCGTTGCGC TCACTGCCCG

4810

CGCGCGGGA CTGCGCTCGG 5040 TTATCCACAG AATCAGGGGA TAACGCAGGA AAGAACATGT GAGCAAAAGG CCAGCAAAAG GCCAGGAACC TTAGTCCCCCT ATTGCGTCCT TTCTTGTACA CTCGITTTTCC GGTCGTTTTC CGGTCCTTGG TICGGACCCC ACGGATTACT CACTCGATTG AGTGTAATTA ACGCAACGCG AGTGACGGGC GTAAAAAGGC CGCGTTGCTG GCGTTTTTCC ATAGGCTCCG CCCCCTGAC GAGCATCACA CATTTTTCG GCGCAACGAC CGCAAAAAGG TATCCGAGGC GGGGGACTG CTCGTAGTGT CCCCCCCT GACGCGAGCC AATAGGTGTC ACTATAAAGA TACCAGGCGT TCCACCGCTT TGGGCTGTCC TGATATTTCT ATGGTCCGCA CCTGCCGCTT ACCGGATACC GGACGCCGAA TGGCCTATGG ATGCTCACGC TGTAGGTATC TCAGTTCGGT GTAGGTCGTT CGCTCCAAGC TGGGCTGTGT GCACGAACCC CCCGTTCAGC AGTCAAGCCA CGTCCTTGGG GGGCAAGTCG TACGAGTGCG ACATCCATAG GGGAAACCTG TCGTGCCAGC TGCATTAATG AATCGGCCAA GAGGCGGTFF GCGTAFTGGG CGCTCTTCCG CTTCCTCGCT CACTGACTCG CTCCGCCAAA CGCATAACCC GCGAGAAGGC GAAGGAGCGA GTGACTGAGC CCATTATCCC 4910 GGTAATACGG 5030 5150 5210 5270 5330 TCGTTCGGCT GCGGCGAGCG GTATCAGCTC ACTCAAAGGC AGCAAGCCGA CGCCGCTCGC CATAGTCGAG TGAGTTTCCG 4900 5080 5140 5260 SAGCTCCCTC GTGCGCTCTC CTGTTCCGAC TTCCGAGGAG CACGCGAGAG GACAAGGCTG CGCTTTCTCA ACCCGACAGG GGAAGCGTGG CGCTTTCTCA CCTTCGCACC GCGAAAGAGT 5200 5380 4890 5130 AGGTGGCGAA 5070 5190 5370 5310 CTCAAGTCAG A TGTCCGCCTT TCTCCCTTCG ACAGGCGGAA AGAGGGAAGC 5120 5180 5360 5300 5170 AAAATCGACG CTTTCCAGTC GAAAGGTCAG 4870 Trececened 5050 5110 AAGGGGGACC 5350

42156

FIGURE 191

5460	5520	5580	5640	5700	5760	5810 5820	5880	5940	6000
AGACACGACT	GTÄGGCGGTG	GTATTTGGTA	TGATCCGGCA	ACGCGCAGAA	CAGTGGAACG	AAGGATCTTC ACCTAGATCC	ACTTGGTCTG	TTTCGTTCAT	TTACCATCTG
TCTGTGCTGA	CATCCGCCAC	CATAAACCAT	ACTAGGCCGT	TGCGCGTCTT	GTCACCTTGC	TTCCTAGAAG TGGATCTAGG	TGAACCAGAC	AAAGCAAGTA	AATGGTAGAC
5450	5510	5570	5630	5690	5750	5810	5870	5930	5990
CAACCCGGTA	AGCGAGGTAT	TAGAAGGACA	TGGTAGCTCT	GCAGCAGATT	GTCTGACGCT	AAGGATCTTC	ATATGAGTAA	GATCTGTCTA	ACGGGAGGCC
GTTGGGCCAT	TCGCTCCATA	ATCTTCCTGT	ACCATCGAGA	CGTCGTCTAA	CAGACTGCGA	TTCCTAGAAG	TATACTCATT	CTAGACAGAT	TGCCCTCCCG
5440	5500	5550 5560 TGGCCTACAC ACCGGATTGA TGCCGATGTG	5620	5680	5740	5800	5860	5920	5980
GTCTTGAGTC	GGATTAGCAG		GAAAAAGAGT	TTGTTTGCAA	TTTCTACGGG	GATTATCAAA	TCTAAAGTAT	CTATCTCAGC	TAACTACGAT
CAGAACTCAG	CCTAATCGTC		CTYTYTYCTCA	AACAAACGTT	AAAGATGCCC	CTAATAG'FIT	AGATTTCATA	GATAGAGTCG	ATTGATCCI'A
5430	5490		5610	5670	5730	5790	5850	5910	5970
GGTAACTATC	ACTGGTAACA		GTTACCTTCG	GGTGGTTTTT	CCTTTGATCT	TTGGTCATGA	TTTAAATCAA	AGTGAGGCAC	GTCGTGTAGA
CCATTGATAG	TGACCATTGT		CAATGGAAGC	CCACCAAAA	GGAAACTAGA	AACCAGTACT	AAATTTAGTT	TCACTCCGTG	CAGCACATCT
5420	5480	5540	5600	5660	5720	5780	5840	5900	5960
CGCCTTATCC	GGCAGCAGCC	CTTGAAGTGG	GCTGAAGCCA	CGCTGGTAGC	TCAAGAAGAT	TTAAGGGATT	AAAATGAAGT	ATGCTTAATC	CTGACTCCCC
GCGGAATAGG	CCGTCGTCGG	GAACTTCACC	CGACTTCGGT	GCGACCATCG	AGTTCTTCTA	AATTCCCTAA	TTTTACTTCA	TACGAATTAG	GACTGAGGGG
5410	5470	5530	5590	5650	5710	5770	5830	5890	5950
CCGACCGCTG	TATCGCCACT	CTACAGAGTT	TCTGCGCTCT	AACAAACCAC	AAAAAGGATC	AAAACTCACG	TTFTAAATTA	ACAGTTACCA	CCATAGTTGC
GGCTGGCGAC	ATAGCGGTGA	GATGICICAA	AGACGCGAGA	TTGTTTGGTG	T'FFTTCCTAG	TYTTGAGTGC	AAAATTFAAT	TGTCAATGGT	GGTATCAACG

FICURE 19J

GCCTCCAGAT TTATCAGCAA

6050

CGGGGTCACG ACGTTACTAT GGCGCTCTGG GTGCGAGTGG CCGAGGTCTA AATAGTCGTT

CACGCTCACC

GCCCCAGING TGCAATGATA CCGCGAGACC

6180 6100 6110 6120 GAAGTGGTCC TGCAACTTTA TCCGCCTCCA TCCAGTCTAT TAATTGTTGC CGGGAAGCTA GAGTAAGTAG TTCGCCAGTT AATAGTTTGC AGGTCAGATA ATTAACAACG GCCCTTCGAT CTCATTCATC AAGCGGTCAA TTATCAAACG TTGTGCAAAA 6360 ATTIGGTOGG TOGGOOTIVC COGCTOGGGT CITICACCAGG ACGITIGAAAT AGGCGGAGGT 6240 6230 6230 6240 GCAACGTTGT TGCCATTG TGCTGTCACG CTCGTCGTTT GGTATGGCTT CGTTCGTTG TGGTTTCACG CTCGTCGTTA GGTATGCTTT CGTAGGCTT CGTTTCCGTAGC ACCACAGTCC GAGCAGAAA CCATACCGTAAC 6250 6290 6300
CATTCAGCTC CGGTTCCCAA CGATCAAGGC GAGTTACATG ATCCCCCATG TTGTGCAAAA
GTAAGTCGAG GCCAAGGGTT GCTAGTTCCG CTCAATGTAC TAGGGGGTAC AACACGTTTT AAGCGGTTAG CTCCTTCGGT CCTCCGATCG TTGTCAGAAG TAAGTTGGCC GCAGTGTTAT TTCGCCAATC GAGGAAGCCA GGAGGCTAGC AACAGTCTTC ATTCAACCGG CGTCACAATA GTAAGATGCT ACTTTAAAAG CACTCATGGT TATGGCAGCA CTGCATAATT CTCTTACTGT CATGCCATCC GTAAGATGCT GTGAGTACCA ATACCGTCGT GACGTATTAA GAGAATGACA GTACGGTAGG CATTCTACGA 6480 6470 6480 1TTCTVIVGAC TGGTGAGFAC TCAACCAAGT CATTCTGAGA ATAGTGTATG CGCCGACCGA AAAGACACTG ACCACTCATG AGTTGGTTCA GTAAGACTCT TATCACATAC GCCGCTGGCT 6550 6550 6560 TCTTCGGGGC GAAAACTCTC AAGGATCTTA CCGCTGTTGA ACGACAACT ACGATCTTA ACCTTTTGCA AGAAGCCCCG CTTTTTCAACAAT GCCGACAACT GTTGCTCTTG CCCGGCGTCA ATACGGGATA ATACCGCGC ACATAGCAGA ACTTTAAAAG CAACGAGAAC GGGCCGCAGT TATGCCCTAT TATGGCGCGG TGTATCGTCT TGAAATTTTC 6170 6350 6160 6340 6400 GCCGAGCGCA 6150 6330 6510 6390 6080 AGCCGGAAGG 6140 6320 6380 TAAACCAGCC 6310 6370

44156

FIGURE 19K

		·							
6660	6720	6780	6840	6900	6950	7020	7080	7140	7200
TTTACTTTCA	GGAATAAGGG	AGCATTTATC	AAACAAATAG	GGAGATCTGC	ACCITITITI TTAATITIAT	GGTCGACTCT	CTYGTGTGTT	GCTTGACCGA	ATGTACGGGC
AAATGAAAGT	CCTTATTCCC	TCGTAAATAG	TTTGTTTATC	CCTCTAGACG	TGGAAAAAA AAITAAAATA	CCAGCTGAGA	GAACACACAA	CGAACTGGCT	TACATGCCCG
6650	6710	6770	6830	6890	6950	7010	7070	7130	7190
TYCAGCATCT	CGCAAAAAG	ATATTATTGA	TTAGAAAAT	CGACGGATCG	ACCTTTTTTT	GATCCCCTAT	CTGCTCCCTG	ACAAGGCAAG	CTGCTTCGCG
AAGTCGTAGA	GCGTTTTTTC	TATAATAACT	AATCTTTTTA	GCTGCCTAGC	TGGAAAAAA	CTAGGGGATA	GACGAGGGAC	TGTTCCGTTC	GACGAAGCGC
6640	6700	6760	6820	6880	6940	7000	7060	7120	7180
CCAACTGATC	GGCAAAATGC	TCCTTTTTCA	TTGAATGTAT	CACCTGACGT	AGCCAGAGTA	CCGATCTCCC	AAGCCAGTAT	TTAAGCTACA	GCGTTTTGCG
GGTTGACTAG	CCGTTTTACG	AGGAAAAAGT	AACTTACATA	GTGGACTGCA	TCGGTCTCAT	GGCTAGAGGG	TTCGGTCATA	AATTCGATGT	CGCAAAACGC
	6690	6750	6810	6870	6930	6990	7050	7110	7170
	AAAACAGGAA	CTCATACTCT	GGATACATAT	CGAAAAGTGC	GGCTTCGAAT	GAGTTTGGCG	CCGCATAGTT	CGAGCAAAAT	TTAGGGTTAG
	TYTYTGTCCTT	GAGTATGAGA	CCTATGTATA	GCTTTTCACG	CCGAAGCTTA	CTCAAACCGC	GGCGTATCAA	GCTCGTTTTA	AATCCCAATC
6620 6630	6680	6740	6800	6860	6920	6980	7040	7100	7160
GATGTAACCC ACTCGTGCAC	TGGGTGAGCA	ATGTTGAATA	TCTCATGAGC	CACATTTCCC	GAGGCGCGCC	TTTTGAGATG	TGCTCTGATG	GAGTAGTGCG	AAGAATCTGC
CTACATTGGG TGAGCACGTG	ACCCACTCGT	TACAACTTAT	AGAGTACTCG	GTGTAAAGGG	CTCCGCGCGG	AAACTCTAC	ACGAGACTAC	CTCATCACGC	TYCTTAGACG
6610	6670	6730	6790	6850	6910	6970	7030	7090	7150
GATCCAGTTC	CCAGCG1"1TC	CGACACGGAA	AGGGTTATTG	GGGTTCCGCG	TAGGTGACCT	TTTATTTTATT	CAGTACAATC	GGAGGTCGCT	CAATTGCATG
CTAGGTCAAG	GGTCGCAAAG	GCTGTGCCTT	TCCCAATAAC	CCCAAGGCGC	ATCCACTGGA	AATAAAATA	GTCATGTTAG	CCTCCAGCGA	GTTAACGTAC

FICURE 19L

CCCATTGACG CAAATGGGCG GTAGGCGTGT ACGGTGGGAG GTCTATATAA GCAGAGCTCT GGGTAACTGC GTTTACCCGC CATCCGCACA TGCCACCCTC CAGATATATT CGTCTCGAGA

TGGGAGTTTG TTTTGGCACC AAAATCAACG GGACTTTCCA AAATGTCGTA ACAACTCCGC ACCCTCAAAC AAAACCGTGG TTTTAGTTGC CCTGAAAGGT TTTACAGCAT TGTTGAGGCG

TGGCCGTGGA TAGCGGTMTG ACTCACGGGG ATTTCCAAGT CTCCACCCCA TTGACGTCAAAACCTCCAAGTTCA GAGGTGGGGT AACTGCAGTT

GTACATCTAC GTATTAGTCA TCGCTATTAC CATGGTGATG CGGTTTTGGC AGTACATCAA CATGTAGATG CATAATCAGT AGCGATAATG GTACCACTAC GCCAAAACCG TCATGTAGTT

TAAATGGCCC GCCTGGCATT ATGCCCAGTA CATGACCTTA TGGGACTTTC CTACTYGGCAATTAACCGGG CGGACCGTAA TACGGGTCAT GTACTGGAAT ACCCTGAAAG GATGAACCGT

CTTGGCAGTA CATCAAGTGT ATCATATGCC AAGTACGCCC CCTATTGACG TCAATGACGG GAACCGTCAT GTAGTTCACA TAGTATACGG TTCATGCGGG GGATAACTGC AGTTACTGCC

GGGACTITICC ATTGACGICA ATGGGTGGAC TATTTACGGT AAACTGCCCA TIGCGGITTAT CCCIGAAAGG TAACIGCAGI TACCCACCIG ATAAAIGCCA ITIGACGGGI

AACGCCAATA

7350 7360 7360 7360 7370 7370 CCCGCCCATT GACGTCAATA ATGACGTATG 1"ICCCATAGT ACCGAC'IGGC GGGTTGCTGG GGGCGGGTAA CTGCAGITTAT TACTGCATAC AAGGGTATCA CCCAACGACC TGCCTGACCG

ATTAGTTCAT AGCCCATATA TGGAGTTCCG CGTTACATAA CTTACGGTAA ATGGCCCGCC TAATCAAGTA TCGGGTATAT ACCTCAAGGC GCAATGTATT GAATGCCATT TACCGGGCGG

CAGATATACG CGTTGACATT GATTATTGAC TAGTTATAA TAGTAATCAA TTACGGGGTC GTCTATATGC GCAACTGTAA CTAATAACTG ATCAATAATT A1CATTAGTT AATGCCCCAG

pD17-hG1b

FIGURE 19M

FIGURE 19N

pD17-hG1b

7810 7850 7860 CTGGCTAACT AGAGAACCCA CTGCTTACTG GCTTATCGAA ATTAATACGA CTCACTATAG GACCGATTGA TCTCTTGGGT GACGAATGAC CGAATAGCTT TAATTATGCT GAGTGATATC

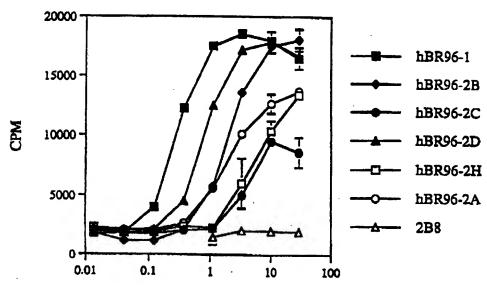
7870 GGAGACCCAA GCTT CCTCTGGGTT CGAA

7880

47156

FIGURE 20

Complement Dependent Cytotoxicity



Concentration IgG (µg/ml)

FIGURE 21

Antibody Dependent Cell-Mediated Cytotoxicity

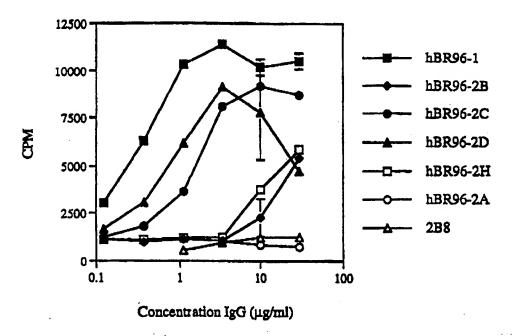
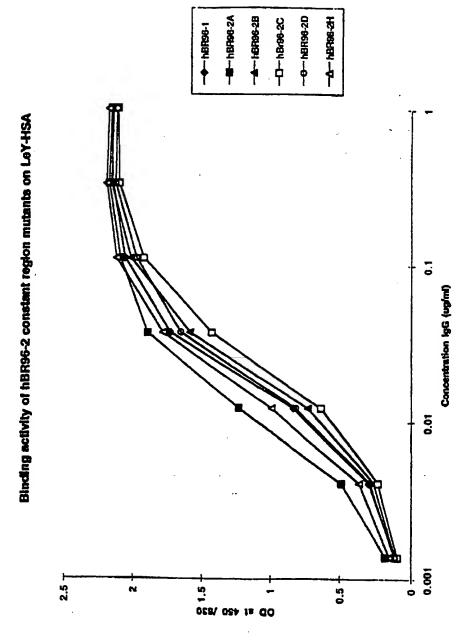


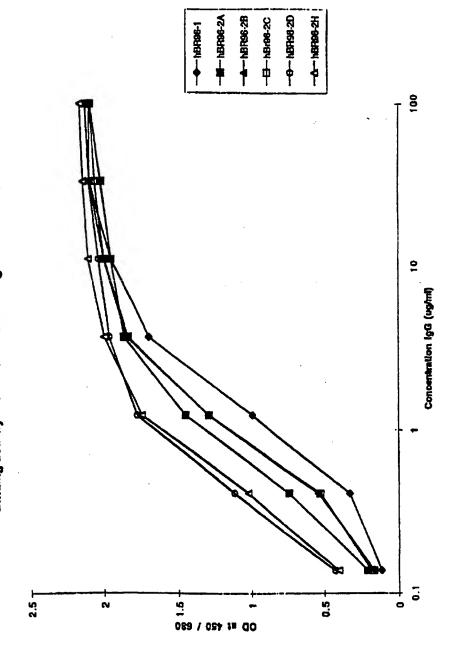
FIGURE 22



50156



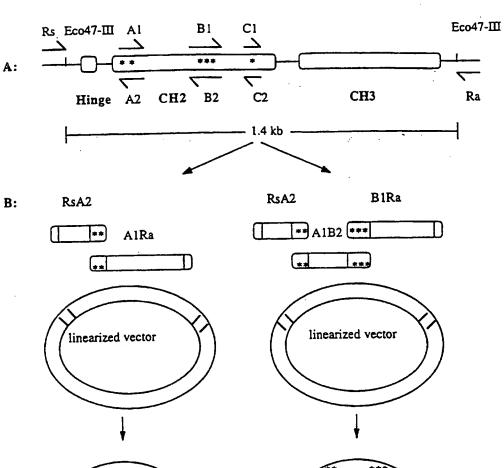
FIGURE 23

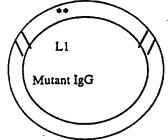


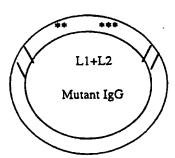
51156



L1 L2 L3







Figur 25

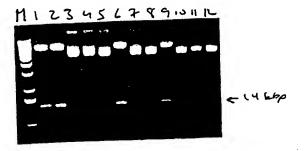


Figure 26

hBR96-2 Heavy Chain Variable Region (VH)

EVQLVESGGG LVQPGGSLRL SCAASGFPFS DYYMYWVRQA PGKGLEWVSY

51 61 71 881 91
ISQDGDITDY ADSVKORFTI SRDNAKNSLY LQMINSLRDED TAVYYCARGL

101 111
ADGAWFAYWG QGTLVTVSS

human IgGI constant

YFPEPVTVSW NSGALTSGVH TFPAVLQSSG LYSLSSVVTV PSSSLCTQTY

ICNVNHKPSN TKVDKKVEPX SCDKTHTCPP CHAPELOGGP SVPLFPPKPK

DTLMISRTPE VTCVVVDVSH EDPEVKFNWY VDGVEVHRAK TKPREEQYNS
310 311 320 311

TYRVVSVLTV LHQDWLNGKED YGOKVSNKAL PAPPLEKTISK AKGGPREPQV

YTLPPSRDEL TKNQVSLTCL VKGFYPSDIA VEWESNGQPE NNYKTTPPVL

DSDGSFFLYS KLTVDKSRWQ QGNVFSCSVM HEALHNHYTQ KSLSLSPGK

Figure 27

hBR96-2A: Heavy Chain Variable Region (VH)

1 21 31 41
EVQLVESGGG LVQPGGSLRL SCAASGFPFS DYYMYWVRQA PGKGLEWVSY

51 61 71 81 91
ISQDGDITDY ADSVKGRFTI SRDNAKNSLY LQMNSLRDED TAVYYCARGL

101 111
-ADGAWFAYWG QGTLVTVSS

hBR96-2A: Human Heavy Chain IgG1 Constant Region ACH2

A STKGPSVFPL APSSKSTSGC TAALGCLVKD YFPEPVTVSW NSGALTSGVH

TFPAVLQSSG LYSLSSVVTV PSSSLGTQTY ICNVNHKPSN TKVDKKVEPK

SCDKTHTCPP CP GQPREPQV YTLPPSRDEL TKNQVSLTCL VKGFYPSDIA

VEWESNGQPE NNYKTTPPVL DSDGSFFLYS KLTVDKSRWQ QGNVFSCSVM

HEALHNHYTG KSLSLSPGK

Figure 28

This sequence is the chi BR96 IgG1 with CH2 deleted.

301 KLTVDKSRWQ QGNVFSCSVM HEALHNHYTQ KSLSLSPGK

1 EVNLVESGGG LVQPGGSLKV SCVTSGFTPS DYYMYWVRQT PEKRLEWVAY
51 ISQGGDITDY PDTVKGRFTI SRDNAKNTLY LQMSRLKSED TAMYYCARGL
101 DDGAWFAYWG QGTLVTVSVA STKGPSVFPL APSSKSTSGG TAALGCLVKD
151 YPPEPVTVSW NSGALTSGVH TFPAVLQSSG LYSLSSVVTV PSSSLGTQTY
201 ICNVNHKPSN TKVDKKVEPK SCDKTHTCPP CHGQPREPQV YTLPPSRDEL
251 TKNQVSLTCL VKGFYPSDIA VEWESNGQPE NNYKTTPPVL DSDGSFFLYS

tntern: at Application No PCT/US 97/13562

PC 6	CATION OF SUBJECT MATTER C12N15/62 A61K39/395 A6 C07K16/30 C07K16/46 C0 C12N5/10 //C07K19/00 International Patent Classification (IPC) or to both nationa			A61K51/10 C12N1/21
. FIELDS S	EARCHED surrentation searched (classification system followed by commentation searched)	dassification sym	bols)	
IPC 6	C07K A61K			
	on searched other than minimum documentation to the ex	tent that such do	ouments are included in t	he fields searched
Documentant	DI SELICIES COLO. C			
Floatennic da	ata base consulted during the international search (name	of data base and	d, where practical, search	terms used)
Election of		•		
C DOCUME	ENTS CONSIDERED TO BE RELEVANT			Relevant to claim No.
Category *	Citation of document, with indication, where appropriate	e, of the relevant	passages	, and the second
Oglagory				1-8,
v	S. GILLIES ET AL.: "Antig	en bindin	g and	23-25
X				25 25
	chimeric antibodies with "	uman tumo)r	
	ficities "			
	HUMAN ANTIBODIES AND HYBRI vol. 1, no. 1, 1990, STONE	HAM. MA.	USA,	
	pages 47-54, XP002050448			
	see the whole document			
		,		ŀ
·		-/		
]				
				1
}				
			•	
1				Ì
			X Patent family men	bers are listed in annex.
X F	urther documents are listed in the continuation of box C.			
* Special	categories of cited documents :	•1	" later document publish	ed after the international filing date at in conflict with the application but
1 .	and defining the general state of the art which is not		oited to understand tr	le bratopie or uterry
	ment certainly are particular relevance nsidered to be of particular relevance ier document but published on or after the international	•	X* document of particular	relevance; the claimed invention
1 Alie	na dete		CARROL DE CONSIDERA	teo when the document is taken alone
	ument which may throw doubts on priority claim(s) or tich is cited to establish the publication date of another	•	Y" document of particular	relevance; the claimed inventors
مفند ا	non a oxed to secondarize reason (as specified) ation or other special reason (as specified) nument referring to an oral disclosure, use, exhibition or		document is combine ments, such combine	d with one or more other such docu- tion being obvious to a person skilled
	her means sument published prior to the international filing date but		in the art. S* document member of	
let	ter than the priority date diagrams			international search report
Date of	the actual completion of the international search	ļ		# 150 THE PERSON NAME OF THE PER
			2 1. 01. 98	
1	17 December 1997			
Name a	and mailing address of the ISA		Authorized officer	
Name a	and mailing address of the ISA European Patent Office, P.B. 5818 Patentiaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo ni,	1	Nooij,	-

Intern ust Application No PCT/US 97/13562

C.(Continue	BOON) DOCUMENTS CONSIDERED TO BE RELEVANT	PCT/US 97/13562
Category *		Relevant to claim No.
X	G. SCHREIBER ET AL.: "An unmodified anticarcinoma antibody, BR96, localizes to and inhibits the outgrowth of human tumors in nude mice." CANCER RESEARCH, vol. 52, no. 12, 15 June 1992, BALTIMORE, MD, USA, pages 3262-3266, XP002050449 see abstract	33,35,36
A		1,2,5,7, 8,11-18, 23
A	A. DUNCAN ET AL.: "The binding site for Clq on IgG." NATURE, vol. 332, no. 6166, 21 April 1988, LONDON, GB, pages 738-740, XP002050450 cited in the application see the whole document	1,2,5,7,
A .	J. LUND ET AL.: "Human FcgammaRI and FcgammaRII interact with distinct but overlapping sites on human IgG." THE JOURNAL OF IMMUNOLOGY, vol. 147, no. 8, 15 October 1991, BALTIMORE, MD, USA, pages 2657-2662, XP002050451 cited in the application see abstract	1,2,5,7,
	Y. XU ET AL.: "Residue at position 331 in the IgG1 and IgG4 CH2 domains contributes to their differential ability to bind and activate complement." THE JOURNAL OF BIOLOGICAL CHEMISTRY, vol. 269, no. 5, 4 February 1994, BALTIMORE, MD, USA, pages 3469-3474, XP002050452 cited in the application see abstract see discussion	1-8
	T. MICHAELSEN ET AL.: "One disulfide bond in front of the second heavy chain constant region is necessary and sufficient for effector functions of human IgG3 without a genetic hinge." PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF THE USA, vol. 91, no. 20, 27 September 1994, WASHINGTON, DC, USA, pages 9243-9247, XP002050453 see the whole document	1,2,5,7,
	-/	1

1

Intern at Application No PCT/US 97/13562

		PC1703 37	
Continu	tion) DOCUMENTS CONSIDERED TO BE RELEVANT	·	Relevant to claim No.
Category *	Citation of cocument, with indication, where appropriate, of the relevant passages		
4	L. TAN ET AL.: "Influence of the hinge region on complement activation, Clq binding, and segmental flexibility in chimeric human immunoglobulins." PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF THE USA, vol. 87, no. 1, January 1990, WASHINGTON, DC, USA, pages 162-166, XP002050454 see the whole document		1-8
A	EP 0 699 756 A (BRISTOL-MYERS SQUIBB COMPANY) 6 March 1996 cited in the application		11-18, 23,25, 28,29, 31-52
	see examples see claims	,	·
		<i>,</i>	
			1
٠			
	·		
	DIL.		
í			

Form PCT/ISA/210 (continuation of second sheet) (July 1992)

International application No. PCT/US 97/13562

Box i Obse	ervations where certain claims were found unsearchable (Continuation of Item 1 of Ilrat sheet)
This Internation	ial Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:
becau	s Nos.: se they relate to subject matter not required to be searched by this Authority, namely: FURTHER INFORMATION sheet PCT/ISA/210
an ext	s Nos.: so they relate to parts of the International Application that do not comply with the prescribed requirements to such ent that no meaningful International Search can be carried out, specifically: FURTHER INFORMATION sheet PCT/ISA/210
3. Claims becaus	Nos.: se they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).
Box II Obse	rvations where unity of invention is lacking (Continuation of item 2 of first sheet)
This Internations	al Searching Authority found multiple inventions in this international application, as follows:
1. As all n	equired additional search fees were timely paid by the applicant, this International Search Report covers all able claims.
2. As all s of any a	earchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment additional fee.
3. As only covers	some of the required additional search fees were limely paid by the applicant, this International Search Report only those claims for which fees were paid, specifically claims Nos.:
\$	
4. No requirestricte	aired additional search fees were timely paid by the applicant. Consequently, this International Search Report is ad to the invention first mentioned in the claims; it is covered by claims Nos.;
Remark on Prot	The additional search fees were accompanied by the applicant's protest. No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

Claims Nos.: 26,27

because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

Claim 26 represents a method of detection/diagnosis and refers forward to claim 30, which represents a method of treatment. Claim 27 refers to a method in claim 24; however, in claim 24 a product is claimed, not a method.

Remark: Although claims 1-22, 25, 28-32 and 34-36 are directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.

		Application No 97/13562				
Patent document cited in search report	Publication date		Patent family member(s)		Publication date	
EP 699756 A	06-03-96	AU CA JP	2834995 2155397 8191692	7 A	15-02-96 05-02-96 30-07-96	
					•	
	· .					
				,	•	
-						
		t				
				•		
,						
	•				*	
•			-			